PATTERN OF REPRODUCTIVE HORMONES IN WOMEN WITH INFERTILITY IN ZARIA, NORTHERN NIGERIA

BY

ISAH IDRIS AINAVI

DEPARTMENT OF CHEMICAL PATHOLOGY,

FACULTY OF MEDICINE

AHMADU BELLO UNIVERSITY ZARIA

OCTOBER, 2009

PATTERN OF REPRODUCTIVE HORMONES IN WOMEN WITH INFERTILITY IN ZARIA, NORTHERN NIGERIA

BY

ISAH IDRIS AINAVI

(MBBS, JOS)

MSC/med/45429/2004 - 2005

A PROJECT REPORT SUBMITTED TO THE POST GRADUATE SCHOOL, AHMADU BELLO UNIVERSITY ZARIA IN PARTIAL FULFILMENT FOR THE AWARD OF MASTERS DEGREE(M.sc) IN CHEMICAL PATHOLOGY.

DEPARTMENT OF CHEMICAL PATHOLOGY,

FACULTY OF MEDICINE,

AHMADU BELLO UNIVERSITY ZARIA.

OCTOBER, 2009

DECLARATION

I declare that the work reported in the thesis entitled “Pattern of reproductive hormones in women with infertility in Zaria, Northern Nigeria” has been performed by me in the Department of Chemical Pathology, Faculty of Medicine, Ahmadu Bello University , Zaria under the supervision of : Prof. H.S. Isah (BSc, MSc, PhD,CChem, FRSC), : Dr. I.S. Aliyu (MBBS, FMCPath) and Dr. A.J. Randawa (MBBS, FWACS). The information derived from the literature has been duely acknowledged in the text and a list of references provided. No part of this project was previously presented for another degree or diploma at any university.

Isah Idris Ainavi

_____	_____	_____
Name of student	**signature**	**date**

CERTIFICATION BY HEAD OF DEPARTMENT

This thesis was submitted to the Department of Chemical Pathology,Faculty Of Medicine, Ahmadu Bello University, Zaria and was accepted as partial fulfilment of the requirements for the award of degree of masters (Msc) in Chemical Pathology.

Dr. I.S Aliyu (MBBS, FMC path)

Senior Lectural/Consultant and Head of Department

CERTIFICATION BY SUPERVISORS

This is to certify that the research work for this thesis titled “Pattern of ReproductiveHormones in Women with infertility in Zaria, Northern Nigeria” and the subsequentpreparation of the thesis was carried out under our supervision.

_____ _____

MAJOR SUPERVISOR DATE
Prof. H.S. Isah (Bsc, Msc, PhD’
CChem, FRSC).
Lectural/Consultant,
Department of Chemical pathology,
Faculty of Medicine ,
Ahmadu Bello University,
Zaria, Nigeria.

_____ _____

SECOND SUPERVISOR DATE
Dr. I.S. Aliyu (MBBS, FMC Path)
Senior Lectural/Consultant,
Department of Chemical pathology,
Faculty of Medicine ,
Ahmadu Bello University,
Zaria, Nigeria.

_____ _____

THIRD SUPERVISOR DATE
Dr. A. J. Randawa (MBBS, FWACS)
Senior Lectural/Consultant,
Department of Obstetics and Gyneacology,
Faculty of Medicine ,
Ahmadu Bello University,
Zaria, Nigeria.

THESIS APPROVAL

This thesis entitled " Pattern of Reproductive Hormones in Women with Infertility in zaria, Northern Nigeria" by ISAH IDRIS AINAVI meets the regulations governing the award of MASTERS DEGREE Ahmadu Bello University, Zaria and is approved for the contribution to knowledge and literacy presentation.

_____ _____

EXTERNAL EXAMINER Date
Prof. O.A. Afonja (MD)
Department of Chemical Pathology,
Lagos University Teaching Hospital,
Lagos, Nigeria.

_____ _____

INTERNAL EXAMINER DATE
Prof. H.S. Isah (Bsc, Msc, PhD'
CChem, FRSC).
Lectural/Consultant,
Department of Chemical pathology,
Faculty of Medicine ,
Ahmadu Bello University,
Zaria, Nigeria.

_____ _____

INTERNAL EXAMINER DATE
DR. I.S. Aliyu (MBBS, FMC Path)
Senior Lectural/Consultant,
Department of Chemical pathology,
Faculty of Medicine ,
Ahmadu Bello University,
Zaria, Nigeria.

_____ _____

INTERNAL EXAMINER DATE
Dr. A. J. Randawa (MBBS, FWACS)
Senior Lectural/Consultant,
Department of Obstetics and Gyneacology,
Faculty of Medicine ,
Ahmadu Bello University,
Zaria, Nigeria.

_____ _____

Dean Post Graduate School DATE
Ahmadu Bello University, Zaria.

DEDICATION

1.This thesis is dedicated to all women of child bearing age who are yearning to have a child of their own.

2.This thesis is also dedicated to the memory of my late maternal grand mother **Inya Okoyi Anaza** under whose love and great care I grew up.

ACKNOWLEDGEMENT

First, and foremost I wish to sincerely register my appreciation and profound gratitudes to Almighty ALLAH(Glory be unto Him) for giving me the opportunity and seeing me through to the completion of this work successfully.

I wish to thank and acknowledge the commitments of my supervisors Prof. H.S Isah, Dr. I.S Aliyu, and Dr. A.J Randawa for their immense constructive critisms, advice, and suggestions through out the period of the study. I sincerely register my immeasurable thanks and gratitudes to them. I wish to state here that no amount of words will be enough to register my appreciation but I do pray may ALLAH (Glory be unto Him) rewards you all in manifolds.

I sincerely express my gratitudes to Dr. S.A Akuyam, the course coordinator, Dr. P.O. Anaja of the department of Chemical Pathology, Ahmadu Bello University Zaria and Dr. Adesuin of the department of Obstetrics and Gynaecology , Ahmadu Bello University Zaria for their immense contribution, moral supports and co-operation towards the success of this work.

I strongly appreciate the contribution and co-operation of the doctors, nurses and record clerks of Obstetrics and Gynaecology Clinic and General Out Patient Department (G.O.P.D) of ABUTH Zaria.

Iam indeed grateful to Dr. Olubenga Oguntunde of the department Community Medicine, ABUTH Zaria for his assistance with statistical analysis. He was never tied of me despite his tight schedules.

I wish to appreciate Dr. I.A. Yahaya of the department of Chemical Pathology, Ado Bayero university Kano for stimulating my interest in academics.

I acknowledge my wife Mrs. Aisha Ozuchu Isah for her love, patience and moral support through out the period of this study.

I wish to appreciate the immense contributions of my course mates and colleagues especially Mall. Bello Muhammad and Alhaji Nasiru Lawal (Pambegua) towards the success of this work.

Finally, I thank all those who consented to participate in this research work without which the dream of this success could not have been realized. I do say thank you all.

ABSTRACT

Aims and objective:

The desire for reproduction is a basic human instinct and it is well known that infertility is one of the psychosocial problems affecting many couples world wide. This study aimed to evaluate the pattern of reproductive hormones in women with infertility in Zaria, Northern Nigeria with a view to assessing the contribution of certain endocrinopathies to infertility in the study population.

This was a crosss- sectional study of women with infertility attending infertility clinic in Ahmadu Bello university teaching Hospital (ABUTH), Zaria.

Subjects and Methods:

Concentration of Leutinizing Hormone (LH), follicle stimulating hormone (FSH), Prolactin (PRL), progesterone and Oestradiol were measured in 120 infertile women and 60 apparently healthy fertile women as control in order to assess the contribution and clinical significance of these hormones in the aetiopathogenesis of infertility in these subjects . The serum hormones were measured by the microwell enzyme linked immunosorbent assay (ELISA) technique based on the non competitive sandwich principle for LH, FSH and prolactin while the serum progesterone and oestradiol were measured based on competitive ELISA principle.

The reproducibility of ELISA kits used for the measurement of the hormone was evaluated with available facilities in the Chemical pathology Laboratory of Ahmadu Bello University Teaching Hospital (ABUTH) Zaria.

Results:

The within-runs assay coefficient of variation were 5.9%, 8.4%, 3.1%, 4.3% and 2.1% for LH, FSH, prolactin, progesterone and oestradiol respectively while the between-runs assay coefficient of variation were 6.2%, 9.0%, 6.9%, 7.3% and 2.7% respectively.

Differences in hormone levels between the groups of fertile and infertile women were assessed by the use of Levene's test.

The mean levels of LH, Prolactin, progesterone, oestradiol and LH/FSH ratio were found to be significantly different($p<0.05$) in the infertile women compared to the control groups. The infertile women had a higher ($p<0.05$) mean concentration of LH, prolactin and LH/FSH ratio (34.8±27.8mIU/ml 30.9±20.3ng/ml and 4±5.8) values than the fertile control groups (12.3± 6.8mIU/ml , 8.7 ±5.6ng/ml and 1.1±0.7).The mean serum progesterone and oestradiol in infertile women (5.3±11.1ng/ml and 188.2±157pg/ml) were significantly lower ($p<0.05$) than in infertile women (17.8± 16.9ng/ml and 366±22.7pg/ml).

There was no significant difference ($p>0.05$) in all the serum hormonal profile in infertile women with primary infertility when compared with those with secondary infertility.

Hormonal abnormalities were seen in 71.7% of infertile women comprising 33.3% polycystic ovarian syndrome with hyperprolactinaemia, 13.3% luteal phase deficiency with hyperprolactinaemia , 13.3% polycystic ovarian syndrome, 2.5% luteal phase defficiency, 4.2%Hypergonadotropic hypogonadism, 1.7%Hypergonadoropic hypogonadism with hyperprolactinaemia, 1.7%Hypogonadotropic hypogonadism and 1.7%Hypergonadotropic hypergonadism. Hyperprolactinaemia was found in 50% and has the highest incidence of all the hormonal disorders among the infertile women studied.

Hyper prolactinaemia was also found to coexist with some other hormonal abnormalities in this study .

Polycystic ovarian syndrome was found in 46.6% of infertile women studied.

The infertile women with normal hormonal levels were 28.3%.

Correlation between the biochemical parameters and the clinical parameters were assessed by the use of Pearson's correlation. There were positive and significant correlations between BMI and serum LH in controls. There were no significant correlation ($p>0.05$) between age and biochemical parameters among the infertile women. However there was a positive correlation ($p<0.05$) between age and serum BMI among the controls.

Conclusions:

The results of this study indicate that hormonal disorders may be responsible for some cases of women with infertility. These results also suggest hormonal disorders that occur in infertile women in Zaria, Northern Nigeria may be treatable.

TABLE OF CONTENTS

Title page.....i

Declaration.....ii

Certification by Head of Department ……...iii

Certification by Supervisors……..…iv

Thesis Approval……..……v

Dedication..... vi

Acknowledgement.....vii

Abstract.....ix

Table of contents.....xiii

List of tables.....xxii

List of figures.....xxiv

Abbreviations.....xxv

CHAPTER ONE

1.0 Introduction......1

1.1 Reproduction.....1

1.2 Female reproductive system.....1

1.3 Fertility Hormones.....4

1.3.1 Gonadotropins (LH and FSH).....4

1.3.2 Prolactin.....4

1.3.3 Oestrogens.....5

1.3.4 Progesterone.....7

1.4 Fertility in women.....7

1.5 Infertility.....8

1.6 The statement of problems.....15

1.7 Objective of the study.....19

1.7.1 General objective.....19

1.7.2 Specific objective.....19

CHAPTER TWO

2.0 Literature Review.....21

2.1 Introduction.....21

2.2 The female reproductive cycle.....21

2.2.1The menstrual cycle.....24

2.2.2 The ovulation process.....28

2.2.3 Conception.....29

2.3 Reproductive Hormones.....30

2.3.1Ovarian sex steroid Hormone secretion….....32

2.3.2 Hypothalamic GnRH secretion.....33

2.3.3 Prolactin.....34

2.3.4 Thyroid stimulating hormone.....36

2.3.5 Leutinizing hormone and follicle stimulating hormone..38

2.3.6 Oestrogen and progesterone.....42

2.3.6.2Oestrogen.....42

2.3.6.2 Progesterone.....44

2.3.7 Testosterone.....47

2.4 Infertility.....48

2.4.1 A Concept of infertility.....48

2.4.2 Causes of infertility.....49

2.4.3 Infertility in Africa.....51

2.4.3.1Infertility in black African women…….....51

2.5 Anovulation.....54

2.6 Amenorrhoea.....56

2.7 Overweight and obesity.....57

2.7.1Impact of obesity on fertility.....58

2.7.2 Endocrine Abnormalities.....60

2.7.3 Obesity and spontaneous ovulation.....62

2.8 Polycystic ovarian syndrome (PCOS).....64

2.8.1 Definition.....64

2.8.2 Pathophysiology.....65

2.9 Underweight.....67

2.9.1 Low body weight.....68

2.9.2 Anorexia nervosa and Bulimia nervosa.....69

CHAPTER THREE

3.0 Materials and Methods.....72

3.1 Study Area.....72

3.2 Sample size determination.....73

3.3 Informed consent.....74

3.4 Ethical considerations.....74

3.5 study population.....75

3.6 Inclusion Criteria.....75

3.7 Exclusion criteria.....76

3.8 Operational definition.....76

3.8.1 Anthropometrical Measurements.....76

3.8.2 Body Mass Index.....77

3.8.3 Biochemical Parameter.....77

3.9 Sampling techniques.....78

3.9.1 Anthropometric Measurement.....79

3.9.1.1 Equipment.....79

3.9.1.2 Measuring procedure.....79

3.9.2 Blood collection and processing.....80

3.10 Hormonal assay.....81

3.10.1 Laboratory materials.....81

3.10.1.1Equipment.....81

3.10.1.2 Reagents.....81

3.10.2 Components of FSH Reagent kit.....82

3.10.3 Components of LH Reagent kit.....83

3.10.4 Components of Prolactin Reagent kit.....85

3.10.5 Components of Oestradiol Reagent kit.....86

3.10.6 Components of Progesterone Reagent kit.....88

3.11 **LABORATORY ANALYTICAL METHODS…**……..……..89

3.11.1 Estimation of Serum FSH.. …….....89

3.11.2 Estimation of Serum LH……..……93

3.11.3 Estimation of Serum Prolactin..…….….95

3.11.4 Estimation of Serum Oestradiol.....99

3.11.5 Estimation of Serum Progesterone…….…....103

3.12 Precsion Study…….……..106

3.13 Statiatical Analysis.....106

3.14 Problems Encountered during the Execution of the study.....107

CHAPTER FOUR

4.0 RESULTS.....108

4.1 Analysis of study Population.....108

4.2 Clinical parameters in infertile women and controls……111

4.3 Perfomance Characteristics and Calibrations of ELISA Techiques for Hormonal Assays.....113

4.3.1 Calibration of the method for serum LH.....113

4.3.2 Precision profiles for LH.....113

4.3.3 Calibration of the method for serum FSH.....116

4.3.4 Precision profiles for FSH.....116

4.3.5 Calibration of the method for serum Prolactin.....119

4.3.6 Precision profiles for Prolactin.....119

4.3.7 Calibration of the method for serum Progesterone.....122

4.3.8 Precision profiles for Progesterone.....122

4.3.9 Calibration of the method for serum Oestradiol.....125

4.3.10 Precision profiles for Oestradiol.....125

4.4 Serum hormonal Profile in infertile women and control ……128

4.5 Serum hormonal profile in infertile women with primary infertility compared to infertile women with secondary infertility……..….128

4.6 Pattern of hormonal profile in infertile women 131

4.7 Relationship between BMI and Biochemical parameters in infertile women and controls.....135

4.8 Relationship between Age and BMI.....136

CHAPTER FIVE

5.1 Discussions 139

5.2 Conclusions 150

5.3 Recommendations.....151

References……..……152

Appendix I.....155

Appendix II ……..... ...156

Appendix III ……..157

Appendix IV ……..160

LIST OF TABLES

Table 4.1: The age group distribution of the infertile women and Controls.

Table 4.2: The frequency distributions of some clinical parameters of the infertile women and controls.

Table 4.3: Clinical parameters of control subjects and infertile women.

Table 4.4: Calibration of the methods for the measurement of concentration of LH on multiskan microplate reader at 450nm.

Table 4.5: Precision profile for measuring serum LH on multiskan microplate reader.

Table 4.6: Calibration of the methods for the measurement of concentration of FSH on multiskan microplate reader at 450nm.

Table 4.7: Precision profile for measuring serum FSH on multiskan microplate reader.

Table 4.8: Calibration of the methods for the measurement of concentration of Prolactin on multiskan microplate reader at 450nm.

Table 4.9: Precision profile for measuring serum Prolactin on multiskan microplate reader.

Table 4.10: Calibration of the methods for the measurement of concentration of Progesterone on multiskan microplate reader at 450nm.

Table 4.11: Precision profile for measuring serum Progesterone on multiskan microplate reader.

Table 4.12: Calibration of the methods for the measurement of concentration of oestradiol on multiskan microplate reader at 450nm.

Table 4.13: Precision profile for measuring serum oestradiol on multiskan microplate reader.

Table 4.14: Biochemical parameters of control subjects and infertile women.

Table 4.15: Serum hormonal profile in infertile women with primary infertility compare to infertile women with secondary infertility.

Table 4.16: Pattern of hormonal profile in infertile women.

Table 4.17: Specific hormonal disorders in infertile women.

Table 4.18: Total percentage of major hormonal disorders in infertile women.

LIST OF FIGURES

Fig 1.1: The female reproductive system.

Fig 1.2: Anatomy of the Ovary.

Fig 2.1: Hypothalamic ovarian axis (HPO).

Fig 2.2: Menstrual cycle overview.

Fig 4.1: Serum LH Calibration curve.

Fig 4.2: Serum FSH Calibration curve.

Fig 4.3: Serum Prolactin Calibration curve.

Fig 4.4: Serum Progesterone Calibration curve.

Fig 4.5: Serum Oestradiol Calibration curve.

Fig 4.6: Scatter plots showing relationship between Body mass index and serum LH in controls.

Fig; 4.7: Scatter plots showing relationship between Age and Body Mass Index in controls.

ABBREVIATIONS AND SYMBOLS

ABUTH	**Ahmadu Bello University Teaching Hospital**
AIDS	**Acquired immune defficiency syndrome**
Ab	**Anti – Body**
Ag	**Antigen**
BBT	**Basal Body Temperature**
BP	**Blood pressure**
CHD	**Congenital Heart disease**
CV	**Coefficient of variation**
CRH	**Corticotrophin-releasing hormone**
CRP	**C-reactive protein**
DHEA	**Dehydrepiandrosterone**
DPB	**Diastolic blood pressure**
et al	**and others**
E_2	**Oestradiol**
ELISA	**Enzyme linked immunosorbent assay**
FSH	**Follicle stimulating hormone**
GnRH	**Gonadotropin releasing hormone**
hCG	**Human chorionic gonadotrophin**
HDL	**High-density lipoprotein**
HIV	**Human immunodeficiency virus**

HPO	**Hypothalamic ovarian axis**
IBW	**Ideal Body weight**
IU	**International Unit**
IGF-1	**Insulin- like growth factor-1**
IGFBP-1	**Insulin- like growth factor binding protein-1**
IL	**Interleukin**
IVF	**In vitro fertilisation**
LDL	**Low-density lipoprotein**
L	**Litre**
LGA	**Local Government Area**
LH	**Luteinizing hormone**
LH-RH	**Luteinizing Hormone releasing Hormone**
mg	**Milligram**
ml	**Millilitre**
mmol	**millimole**
n	**Number of estimations**
ng	**Nanogram**
pg	**Picogram**
PHCN	**Power Holding Company of Nigeria**
PIF	**prolactin inhibiting factor**
POF	**Primary Ovarian Failure**

Prl	**Prolactin**
r	**Coefficient of correlation**
SBP	**Systolic Blood Pressure**
SD	**Standard Deviation**
SHBG	**Sex hormone binding globulins**
STD	**Sexually transmitted disease**
TNF	**Tumour necrosis factor**
TRH	**Thyrotrophic-releasing hormone**
TSH	**Thyroid-stimulating hormone**
TSH-RH	**Thyoliberin**
t	**Student t-test**
USA	**United States of America**
WHO	**World Health organization**
º	**Degree**
=	**Equal to**
>	**Greater than**
<	**Less than**
%	**Per cent**
/	**Per**

CHAPTER ONE

1.0 **INTRODUCTION**

1.1 Reproduction

Reproduction is one of the most important functions of the living organism. Reproductive function in women requires the hormones of the hypothalamic- Pituitary-gonadal axis as well as the adrenal glands. The reproductive hormones that regulate the female sexual life are produced by the ovary under the control of the hypothalamic pituitary system.

1.2 Female reproductive system

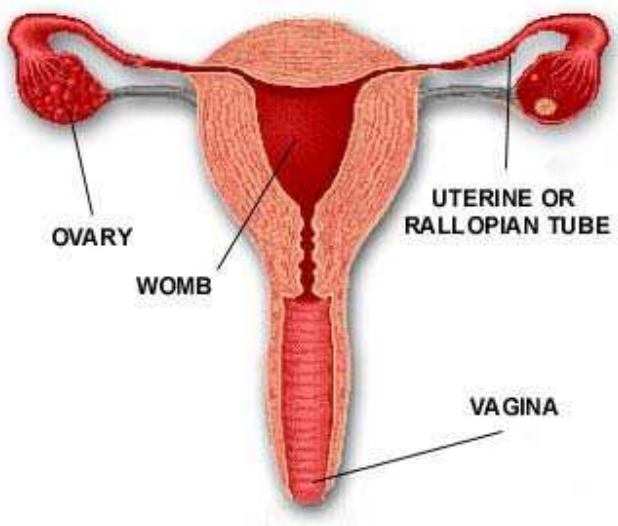


Fig:1.1 The female reproductive system.

The female reproductive system consists of a vagina, uterus, and bilateral fallopian tubes and ovaries. The ovaries are located on either side of the uterus in close proximity to the fallopian tubes. They function as both the producer of ova and the secretors of the sex hormones progesterone and oestrogens. The ovaries have three regions: an outer cortex containing oocytes in various stages of maturation, an inner medullar containing stromal cells and steroid producing cells, and a hillum, which serves as the entrance for nerves and blood vessels and connects the ovary to mesenteric tissue in association with fallopian tubes.

Every healthy female neonate possesses approximately four hundred thousands(400,000) primordial follicles, each containing an immature ovum. During the reproductive life span of an adult woman, 300 to 400 follicles will reach maturity(depol, *et al* 1991, Thalavard, J. C. 1994).

As the ovum matures, it progress from a primordial follicle through primary, secondary, and tertiary follicular phases. A single mature follicle (Graffian follicle) is produce during each normal menstrual cycle at approximately day fourteen. Sorrounding the oocyte of the mature follicle are three distinct cell layers: the *theca externa,* the *theca interna* and *granulosa* cells. The *theca interna* cells are the primary source of androgens which are transported to the adjacent *granulosa* cells, where they aromatised to oestrogens (Thalavard, J.C 1994).

The mature follicle undergoes ovulation by the process of rupture, thereby releasing the oocyte into the proximity of the fallopian tubes. The follicle then fills with blood to form the *corpus heamorragium*. The granulosa and theca cells of the follicle lining quickly proliferate to form lipid-rich luteal cells, replacing the clotted blood and forming the corpus luteum (yellow body). The luteal cells produce oestrogens and progesterone. If fertilisation and pregnancy occur, the corpus luteum persists and continues to produce oestrogen and progesterone. If no pregnancy occurs, the corpus luteum regresses and is eventually replaced by scar tissue.

The fallopian tube arise from the uterus and extend toward the ovaries. They convey the the sperm upward from the uterine cavity and provide the site of fertilisation of the oocyte.The fertilised egg is transported back along the fallopian tubes to the uterine cavity. The uterine cavity is lined by endometrium.The endometrium undergoes cyclical changes in preparation for implantation and pregnancy. During the luteal phase, the endometrial lining increases in thickness and vascularity; during menstruation,the endometrium is shed.

1.3 FERTILITY HORMONES

1.3.1 Gonadotropins: Luteinizing Hormone(LH) and Follicle Stimulating Hormone (FSH)

The primary pituitary hormones that regulate the ovarian functions are LH and FSH. Both hormones were named on the basis of their effects on ovarian function before their effects on testicular function was recognised. LH and FSH are secreted by the same basophilic cells in the pituitary. They are glycoproteins composed of two polypeptide chains designated alpha and beta. The alpha subunits of the hormones are identical and similar to all other pituitary hormones e.g TSH while biologic and immunologic activities are conferred by the unique beta subunit (Vaituaits *et al,*1976). Both subunits are required for endocrine action. In addition, various sugar are linked to these hormones (oligosaccharides and sialic acid residues) in a process termed glycosylation or glycation. The type and number of sugars differ between FSH and LH. Twenty different forms of LH and FSH have been identified based on electrophoretic separation (Vaitukaits *et al,* 1976).

1.3.2 PROLACTIN.

Human prolactin is a single chain polypeptide with a molecular weight of 22,000 dalton(d). It is secreted from the anterior pituitary. Release of prolactin is controlled by a complex neuroedocrine reflex initiated by a

tactile stimulus and regulated by hypothalamic releasing factor i.e Prolacting Releasing factor(PRF) and inhibition factors i.e Prolacting Inhibiting Factor(PIF).

The primary function of prolactin is to initiate breast development and to stimulate and maintain lactation. Unlike FSH and LH prolactin is under tonic inhibitory control of the hypothalamus. The important mediator of inhibition is dopamine and any abnormality in the synthesis, transport or interaction of dopamine diminishes its inhibitory activity and therefore increases prolactin release(Hulting *et al,* 1985).

Prolactin levels are important for normal libido. No significant hypogonadal effects have been observed with low serum prolactin levels. In females, clear pathophysiologic states have been ascribed to hyperprolactinaemia (Hulting *et al,*1989). Most evidence suggest that hyperprolactinaemia abolishes gonadotropin pulsatility by interfering with episodic GnRH release.

Prolactin levels are elevated in renal disease, hypothyroidism, stress, exercise and hypoglycaemia.

Prolactin concentrations may also be raised by drugs such as reserpine oestrogen and lowered by bromocryptine and L-dopa.

1.3.3 OESTROGENS.

Oestrogens are sex hormones that are responsible for the development and maintenance of the female sex and female secondary sexual organs

characteristics. In conjunction with progesterone, they also participate in the regulation of the menstrual cycle and breast and uterine growth and in the maintenance of pregnancy.

Oestrogens are derivatives of the parent hydrocarbon oestrane, which is an 18 -carbon molecule with an aromatic ring A and a methyl group at C-13 (Adashi, E.Y, 1992, and Caro, J 1997).

Oestrogens possess a phenolic hydroxyl group at C-13, which gives the compound an acidic property, and may possess a ketone (oestrone) or hydroxyl group(oestradiol) at C-17 and frequently at C-16(oestriol).

In normal women, most oestrogens are secreted by ovarian follicles and the corpus luteum and during pregnancy, by the placenta. Adrenal glands and testes are also believed to secrete minute quantities of oestrogens. Over 20 oestrogen compounds have been identified, but only 17 beta- oestradiol (E_2), oestrone (E_1), and oestriol (E_3), are known to have any clinical importance.

Oestrogens are also produced by peripheral aromatisation of androgens, primarily androstenediol.

The most potent oestrogen secreted by the ovary is 17beta- oestradiol, because it is derived almost exclusively from the ovaries and its measurement is often considered sufficient to evaluate ovarian function.

Deficiency of oestrogen results in irregular and incomplete development of the endometrium.

1.3.4 PROGESTERONE

Progesterone like oestrogens, is a female sex hormones. In conjuction with oestrogens , it helps to regulate the accessory organs during the menstrual cycle (Adashi, E. Y. and Yen, S.S.C.1999). This hormone is especially important in preparing the uterus for the implantation of the blastocyst and in maintaining pregnancy. In non pregnant women, progesterone is secreted mainly by the corpus luteum. During pregnancy the placenta becomes the major source of this hormone. Progesterone is 21carbon compound containing a keto group (C-3) and a double bond between C-4 and C-5; both structural characteristics are essential for progestational activity.

Initiation and control of luteal secretion of progesterone are regulated by LH and FSH. Deficiency of progesterone results in failure of implantation.

1.4 FERTILITY IN WOMEN

Fertility denotes the actual production of live offspring. In women, fertility means, the ability to become pregnant and have a baby. A woman's reproductive years begin when she starts her menstrual cycles during puberty (about age 13years). The ability to have a child usually ends around age 45years. Though it is potentially possible for a woman to get pregnant until her periods end with menopause (about age 51year).

When a baby girl is born, she already has about four hundred

thousand (400,000) immature eggs (oocytes). These are stored in her ovaries in tiny fluid like sacs called follicles. Once she enters her reproductive years, she starts having monthly cycles. During each cycle, the ovary releases one egg (or less commonly, more than one), which may go on to join with a man's sperm cell and begin a pregnancy. The development and release of the egg depend on a delicate balance of reproductive hormones. Some of these hormones are produced in the ovaries. Others comes from two glands in the brain, the hypothalamus and pituitary.

1.5 INFERTILITY

In Africa, children are the fabric of any society, without which no meaningful social and economic progress is considered worthwhile. According to the World Health Organization (WHO), infertility is defined as the inability to establish a pregnancy within a specified period of time usually one year in couples of child reproductive age who are having regular sexual intercourse without contraception (Fertil 1987:WHO). Infertility also includes the inability to carry a pregnancy to the delivery of a live baby. Infertility is primary if a pregnancy has never occurred, and secondary if there has been a preceding pregnancy, irrespective of the outcome of the pregnancy.

Generally, 15% of couples are said to be infertile, males and females each are believed to separately account for approximately 40% of infertility and are jointly responsible for 20% of cases (Contemporary Ob/Gyn. 1980).

In USA about 13.9% of married potentially fertile couples have infertility problem at some time in their lives (Nandi E *et al*,1993; Rowel PJ;1988 Hillis SD,et al1993) and in the United Kingdom the prevalence of infertility is reported to be 22% of all the patients attending the gynaecology clinic (Fertil Steril;1987;Foster *et al*1989).

WHO in 1991 estimated that between 8 and 12% of couples experienced some form of infertility during their reproductive lives, thus affecting 50 to 80 million worldwide, out of which 20 – 35 million couples in Africa are expected to experience this problem. This can be extrapolated to 3 - 4 million Nigerian couples suffering from infertility (Thomas 1995). However an estimate of infertile couples in Ile-Ife has been put at 19% by Okonofua in 1995, although authors in previous studies in the other parts of Nigeria gave ranging estimates.

In Africa, its prevalence is particularly high in sub-sahara Africans ranging from 20% to 60% (Ogunniyi, 1999).

Infertility is a world wide problem and a life crisis which imposes a profound emotional stress on the individual and couple and creates social strains with friends, family and colleagues.

Unfortunately, its incidence is gradually assuming an alarming proportion in many parts of the developing world. Even though new techniques of diagnosis and treatment have been developed in the industrialised parts of the world, the benefit of these discoveries will take some time to be realised in areas where they are needed most.

In developed countries, infertility affects about 10 to 15% of married couples. In Nigeria, the incidence varies from 20 to 30%. Despite the growing concern about overpopulation and under nutrition in Africa, it is estimated that about 30 to 40% of women in sub sahara Africa would complete their reproductive years without having a child.

The causes of infertility among couples are numerous and are distributed evenly among male and female factors (Pilgrim, 2001). The causes of infertility according to Talbert, 1992; Sharara &McClamrock, 2000 include:

i)Infections(PID)

ii)Tubal factors

iii) Endometriosis

iv) Male factors

v) Ovulation factor

vi) Unexplained infertility

vii) Luteal-phase defect

viii) Cervical factor

ix) Uterine factor

One of the most common causes of infertility in women is pelvic Inflammatory disease (PID). Half of the most frequently reported infections are sexually transmitted diseases (STD's) (Pilgrim, 2001).

In a study undertaken by the World Health Organization (WHO), the cause of infertility in African countries could be attributed to infection secondary to sexually transmitted diseases and pregnancy complications (Dyer *et al*, 2002a).

A recent study in the USA, has shown that African American women presented with a longer duration of infertility and a higher incidence of tubal disease which commonly follows pelvic inflammatory disease(PID) than their white counterparts (Sharara & McClamrock, 2000). In general, tubal disease is most common in developing countries and in poor social economic groups where medical services are not readily available (Willocks & Neilson, 1991).

Another frequent finding in African American women is uterine anomalies such as fibroids. Fifty to seventy five per cent of all African American women are affected with fibroid tumours (Pilgrim, 2001).

Infertility due to endocrine dysfunction was previously thought to be uncommon in African women but with the introduction of immunoassay and other sophisticated technologies in the laboratory practice in Africa, this problem has been found to constitute an important cause of infertility in African women (Rebar *et al,* 1990 and Ujah *et al,* 1991).

Various ovulatory disorders ranging from luteal phase deficiency to anovulation are estimated to account for up to10 to 15% of all infertility problems in Africa (Contemporary obs/Gyn 2003).

Recent advances in endocrinology points to the fact that the hypothalamo-pituitary-ovarian (HPO) axis is essential for the smooth operation of the mechanism of the endocrine function which leads to ovulation and priming of the endometrium for implantation of the zygote which has to pass through a normal fallopian tube following fertilisation (Iris *et al,* 2003).

Ovulation may be evaluated by various means such as basal body temperature (BBT) charts, endocrine evaluation, endometrial biopsy and serial ultral sound. Unfortunately basal body temperature (BBT) charts lacks sensitivity (Bauman J.F.1981).

Endocrine evaluation and endometrial biopsy are more reliable methods of assessing ovulation function (Fox 1980). When endometrium is dated, functional abnormalities of the pituitary-ovarian-endometrial axis may be

detected as well as other endometrial causes of infertility such as infection/endometritis. However some authors have reported that the place of endometrial biopsy in the diagnosis of infertility is doubtful because of inconsistency of their findings, errors in interpretations, and hazard of pregnancy during the luteal phase as well as the limited therapeutic benefit of the procedure.

Fertility in women is tightly regulated by the hypothalamo- pituitary-ovarian (HPO) axis. Any derangement of hypothalamic-pituitary-ovarian(HPO) axis results in menstrual irregularities and ovulation disorders with consequent infertility (Karlo 2003).

There are four major hormonal markers that characterise the menstrual cycle, two are of pituitary origin, which are luteinizing hormone (LH), follicle stimulating hormone (FSH) while the other two are of ovarian origin, which are oestradiol and progesterone (Ferin *et al,* 1993).

The hypothalamic-regulatory hormones which orchestrate the activities of the anterior-pituitary that relates to fertility are gonadotrophin releasing hormones and prolactin inhibitory factor(PIF) or dopamine. The gonadotrophin releasing hormone is a decapeptide which is release in a pulsatile fashion to stimulate release of the gonadotrophins; follicle

stimulating hormone (FSH) and Leutinizing Hormone from anterior pituitary (Willocks and Nelson 1991).

The circulating levels of most major reproductive hormones have been shown to fluctuate, often quite dramatically. This accounts for the large variations shown by individual's cycle even among succession menstrual cycle in the same woman (Ferin *et al*, 1993).

Clues to some hormonal problems may be pointed out by history taking and general physical examination of the female patient. For example, the presence of hirsutism may indicate androgen excess. Hirsutism in conjunction with obesity may be associated with polycystic ovarian syndrome or Cushing's syndrome.

Irregular cycles in association with a low body weight may indicate hypogonadotrophic ovulation problem, as seen in women with eating disorder (anorexia nervosa). A history of galactorrhoea may indicate the presence of hyperprolactinaemia and even the presence of prolactinoma (Brugh *et al*, 2000). A history of hypo or hyperthyroidism may suggest an associated ovulatory problem.

Women who are obese have high incidence of amenorrhoea and infertility. It was found that in the United States of America (USA), 12 % of infertility is caused by female body weight disorders. More than 70% of these women

conceive spontaneously if their weight is corrected through a weight-reducing diet as appropriate (Bates, 1992).

Women in an ealier study in America with presumed anovulation were more than 18kg heavier than obese women with no menstrual abnormalities (Bates,1992).

However some reseachers have claimed that the high basal metabolic Index (BMI) has no adverse effects in black women while others documented a significant decrease in both the implantation rate and pregnancy in overweight women compare to those with a normal body weight (Sharara and Mcclamrock, 2000). Also the percentage of women in the USA with menstrual disorders increases as their percentage of weight above ideal body weight increased (Bates, 1992).

Although this study specifically addresses infertility in women, it is equally important for the male partner to be tested at the same time.

1.6 STATEMENT OF PROBLEM

The desire for reproduction is a basic human instinct. Therefore, infertility causes distress to many couples. The causes of infertility vary from country to country and different social groups (Willocks and Meilson 1991).

In Africa, infertility is a major reproductive health problem with regional prevalence rates of 30-40% (Dyer *et al*, 2002).

Although infertility is not a disease in the classic sense, it is an extremely important personal concern for many couples and a significant health problem for the medical profession.

The psycho-social consequences of infertility include stress, anxiety, depression, marital difficulties, physical violence and social discrimination. Available evidence suggests that the social consequences of infertility are particularly profound for African women as compared to men (Inhorn, 1994a,b).

Regardless of the medical cause of infertility, women receive the major blame for the reproductive setback and they suffer personal grief and frustration, social stigma, ostracism and serious economic deprivations. In Cameroon, Feldman-Savelsberg (1994) reports that infertility is a ground for divorce among the Bangangte tribe causing a woman to lose her access to land distributed by her husband. Where she is able to avoid divorce, an infertile woman receives fewer gifts from her husband and is abandoned in old age with no child to till the land for her. In Egypt, women go through a complicated ritual known as *kabsa* (a form of fertility-producing, polluting boundary violation) in efforts to overcome infertility (Inhorn 1994c). Among the Ekiti of southwestern Nigeria, Ademola (1982) reports that infertile women are treated as outcasts and their bodies are buried on the outskirts of

the town with those of demented persons.

In Nigeria, infertility is the leading reason for gynaecological consultation (Okonofua, 1996) and couples seeking help can place a heavy burden on limited health care resources.

Recent studies from various parts of Africa have demonstrated higher rates of human Immunodefficient (HIV) infection in infertile women compared to fertile controls (Falgusa. E, *et a,l* 1999), suggesting a greater vulnerability of infertile women to the social factors that predispose to HIV.

The fear of infertility has also been identified as the most important reason that women often do not use contraceptives, believing that these could lead to infertility (Otolde V. and Okonofua, 2000).

Until recently studies have focused predominantly on patients in industrialised countries, while the experience of infertility in the developing world has received comparatively little attention (Dyer *et al*, 2002b).

Unlike many other causes of infertility in black community especially in Zaria hormonal factor has been least considered , researched, or even discussed among professionals within the health care community.

However, an aspect of assessment (evaluation) of the contribution of certain endocrinopathies in infertile women is neglected in the management of

patients in most of the Nigerian Hospitals including ABUTH, Zaria, as much attention is being paid to tubal factor. And there is paucity of data on endocrinopathologic contribution in infertile women attending infertility clinics in and around Zaria.

The reported studies on this aspect of infertility were carried out in Kano but in a very limited scope. In view of it's clinical importance in the effective management of infertility there is therefore the need to carry out a study on the pattern of reproductive hormones in infertile women seen in ABUTH, Zaria. This could be of help in the diagnostic, therapeutic and prognostic purposes including provision of assisted reproductive techniques for infertile women and therefore, reduce the emotional stress and social strains on individuals and couples and bring a considerable impact on reproductive health in Zaria.

1.7 OBECTIVES OF THE STUDY

1.7.1 General objective:

The general objective of the study was to evaluate the pattern of reproductive hormones in women with infertility in Zaria, Northern Nigeria with a view to assessing the contribution of certain endocrinopathies to infertility in the study population.

1.7.2: SPECIFIC OBJECTIVES.

The specific objectives of this study were as follows:

1. To measure serum LH, FSH, LH/FSH ratio, prolactin, progesterone and oestradiol in infertile women and apparently healthy fertile women of reproductive age group (controls).
2. To compare the results of serum prolactin, LH, FSH, LH/FSH ratios prolactin, progesterone and oestradiol concentrations obtained from infertile women with those of controls.
3. To measure the anthropometric values (weight, height, body mass indicies) in infertile women and controls.

4. To compare the anthropometric values obtained from women with infertility and those of the control.
5. To determine the association between anthropometric values and reproductive hormone levels in infertile women and controls.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

The normal reproductive cycle in women consists of a sequence of events that are very well co-ordinated.The normal reproductive cycle in women and the hormones involved in reproduction are very crucial in discussing the concept of infertility, as well as those factors that are related to infertility. The body weight has a significant impact on infertility and those hormones specifically related to body weight.

2.2 The female reproductive cycle

In the female reproductive cycle, ovulation is followed by menstrual bleeding in a recurring, predictable sequence, if conception does not occur (Beckmann *et al.*, 1995).

The dynamic relationships between the different components of the reproductive axis in the adult female are such that this reproductive process occurs in cyclic fashion, in an orderly sequence of events. This sequence involves a remarkable co-ordination between hormonal secretion and morphological changes in various organs (Ferin *et al*,1993).

This recurring sequence is established at puberty and continues until the time

of menopause at around age fifty. A woman, therefore, has approximately 30 years of optimal reproductive function. In healthy women, reproductive cycles occur at about 28-day intervals, and most women ovulate 13 to 14 times per year, unless ovulation is interrupted by pregnancy, lactation or oral contraception (Beckmann *et al*, 1995).

The reproductive cycle of the female can be divided into three stages (Ferin *et al*, 1993):

1) the follicular phase, the time for follicular growth;

2) the ovulatory period, when final maturation of the oocyte and its release into the reproductive tract occurs; and

3) the luteal phase, when a newly formed corpus luteum secrets hormones in preparation for implantation.

If the egg is not fertilised and implantation does not occur, a new cycle is initiated as soon as the activity of the corpus luteum wanes. If the fertilised egg implants itself in the uterus, the luteal phase is prolonged and becomes the progestational phase of the pregnancy that follows. Ovulation is induced by the sudden release of large amounts of gonadotrophins from the pituitary gland.

Ovarian steroids also promote sexual receptivity. Corpus luteum development then follows ovulation spontaneously (Ferin *et al.*, 1993).

The reproductive cycle depends on the cyclic interactions between hypothalamic gonadotrophin-releasing hormone (GnRH), the pituitary gonadotrophins follicle stimulating hormone (FSH) and luteinizing hormone (LH), and the ovarian sex steroid hormones oestradiol and progesterone. Through positive – and negative – feedback loops, these hormones stimulate ovulation, facilitate implantation of the fertilised ovum, and bring about menstruation. If any one (or more) of the above hormones becomes elevated or suppressed, the reproductive cycle becomes disrupted and ovulation and menstruation cease.

In the case of female reproductive dysfunction, it is essential to identify which hormones are either elevated or reduced (Beckmann *et al*, 1995).

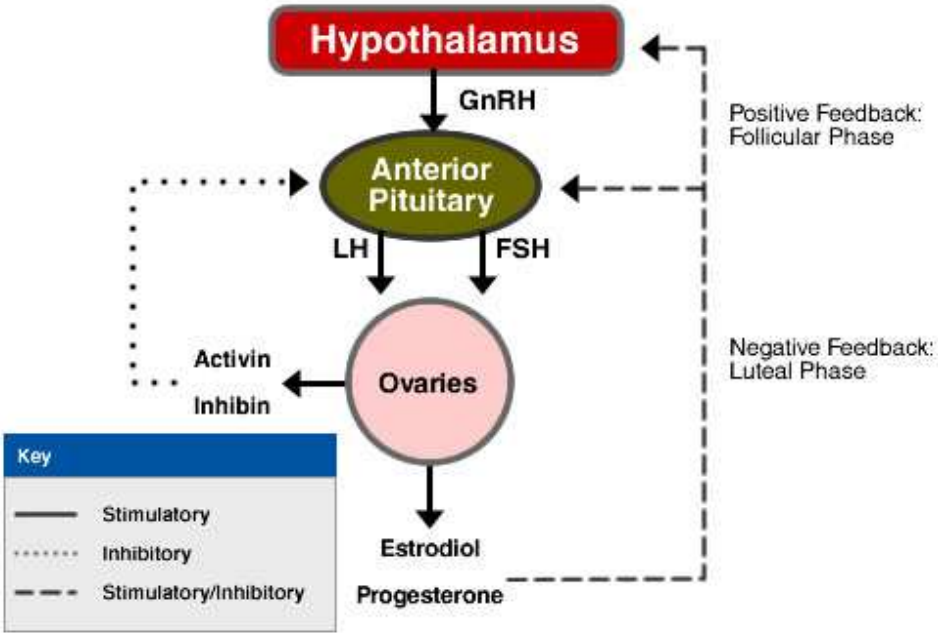


Fig 2.1: Hypothalamic ovarian axis (HPO)

2.2.1 The menstrual cycle

The first half of the endometrial cycle, before ovulation, is characterized by epithelial and stromal differentiation. During the first half of the post-ovulatory phase, specific changes occur in the endometrial epithelium. In the second half, the histological changes affect the stroma, leading to a predicidual reaction. If fertilisation fails to occur, the stromal reaction regresses and menstruation starts. If pregnancy occurs, the endometrial changes progress to formation of the deciduas (Borini & Asch, 1993).

The first phase of the cycle is the menses. The onset of menstrual bleeding by convention is termed the first day of the cycle. The follicular phase

follows menses. Pulsatile GnRH secretion from the hypothalamus results in release of LH and FSH from the anterior pituitary. Follicles are recruited during this period and granulosa cells surrounding the developing oocytes produce oestrogens. Eventually a dominant follicle develops. The oestradiol produced by the developing follicles causes an orderly endometrial proliferation within the uterus during this stage of the menstrual cycle (Brugh *et al.*, 2002).

The pre-ovulatory phase is the third phase of the cycle. During this brief phase, an LH surge occurs, following an oestradiol surge. This triggers ovulation, which occurs 24 to 36 hours after the LH surge. During ovulation, the dominant follicle ruptures, releasing the mature egg. Following ovulation, the final or luteal phase of the cycle is characterised by secretion of progesterone from the corpus luteum (ruptured follicle).

Progesterone is necessary to maintain the endometrium for the impending implantation of an embryo, which occurs most commonly around 5 days after ovulation if fertilisation has occurred. If the corpus luteum is not supported by human chorionic gonadotrophin (hCG), produced by an implanted embryo, it will involute (Brugh *et al.*, 2002). Once the corpus luteum is involuted and if pregnancy does not occur, menstruation begins, and the cycle repeats (Brugh *et al.*, 2002; Beckmann *et al.*, 1995).

The normal ovulatory menstrual cycle can be summarised as follows (Ferin *et al.*, 1993):

1. The menstrual cycle is initiated when conditions allow for the preferential release of FSH. After that, a cohort of follicles is recruited and the follicular phase starts.
2. FSH promotes the conversion of androgens to oestrogens. Oestradiol concentrations increase slowly within the follicles and in the peripheral circulation.
3. Within a few days, one of the follicles in the cohort becomes dominant, increasing oestradiol concentrations and decreasing LH and FSH pulse amplitude. Diminished gonadotrophin concentration slows down the growth of all cohort follicles, except for the dominant one.The dominant follicle then continues to grow.
4. The dominant follicle acquires LH receptors. LH stimulates the synthesis of androgens. These androgens are metabolised into oestrogens. Oestradiol is highly mitogenic and potentiates several local growth factors; thus, the selected follicle will grow rapidly and in a few days become a fully mature Graafian follicle.
5. Maturity of the Graafian follicle is marked by high circulating concentrations of oestradiol, the signal to the hypothalamus and

pituitary gland that the follicle is ready for the ovulatory signal. The long loop oestradiol positive feedback is activated and as result, the gonadotrophin surge occurs.

6. The high gonadotrophin concentrations during the surge arrest granulosa cell proliferation and secretory activity in the Graafian follicle. Oestradiol secretion declines rapidly. Granulosa cells begin to luteinize, and as a consequence, a small pre-ovulatory rise of progesterone occurs.

   Ovulation, the release of the fully grown oocyte, occurs about 18 hours following the gonadotrophin “peak”, or at least 36 hours after the “initiation” of the surge. At ovulation, the oocyte resumes meiosis.

7. After the release of the ooctye, the granulosa layer becomes vascularised, and the granulosa cell completes the process of luteinization, whereby it acquires *de novo* steroid synthesis capacity that it previously lacked. LH stimulates progesterone and oestradiol secretion for this newly formed structure, the corpus luteum. Progesterone in combination with oestradiol, in turn, activates the hypothalamic opiate centre, the result of which is to decrease gonadotrophin pulse frequency.

8. The corpus luteum is a transient organ that has an inherent 12 –14days life span. Thus, estradiol and progesterone secretion peaks about seven to nine days after its formation.
9. Luteolysis, the process of regression of the corpus luteum, results in a rapid decline in progesterone and estradiol concentrations. This leads to menstruation.
10. Through a combination of several factors, which may include the long period of decreased pulse frequency during the luteal phase, a decrease in inhibin secretion and/or of oestradiol and progesterone secretion, FSH concentrations increase relative to those of LH, and a new cycle is initiated.

In the majority of women, the menstrual cycle lasts between 25 and 30days, with the distribution within the range skewed toward cycles with a 28 – 30 day length. The onset of menstruation delineates the termination of an endometrial cycle and the beginning of a new one (Ferin *et al.*, 1993).

2.2.2 The ovulation process

Ovulation is the end process of a series of events initiated by the gonadotrophin surge and resulting in the release of a mature fertilisable egg from a Graafian follicle.

Unfortunately, the precise sequence of local events within the follicle that

lead to rupture of the follicular wall and expulsion of the egg is not known. There is no question that the process of ovulation is initiated by the gonadotrophin surge, which occurs in response to the long loop oestradiol positive feedback, the signal to the brain and pituitary that the dominant follicle has attained maturity. The gonadotrophin surge terminates oestradiol synthesis; the theca cell now changes from an androgen to a progesterone-secreting tissue. Vascular changes in the pre-ovulatory follicle occur within minutes of the LH surge. The multi- layered capillary plexus within the theca dilates causing hyperaemia, a prelude to the ovulatory process. About six hours into the LH surge, there is increasing ovarian blood flow due to decreased vascular resistance, increase in capillary and venule permeability leading to an increase in interstitial fluid volume (Ferin *et al.*, 1993).

2.2.3 Conception

Menstruation does not take place if pregnancy occurs. The oocyte which is released by the Graafian follicle at the time of ovulation is gently swept into the lumen of the fallopian tube by the finger-like structures at the ends – the fimbrae. Ciliated cells in the tubal lumen (the endosalpinx) waft the egg onwards to the ampulla of the tube, which is where fertilisation occurs by the union of egg and sperm (Willocks & Neilson, 1991).

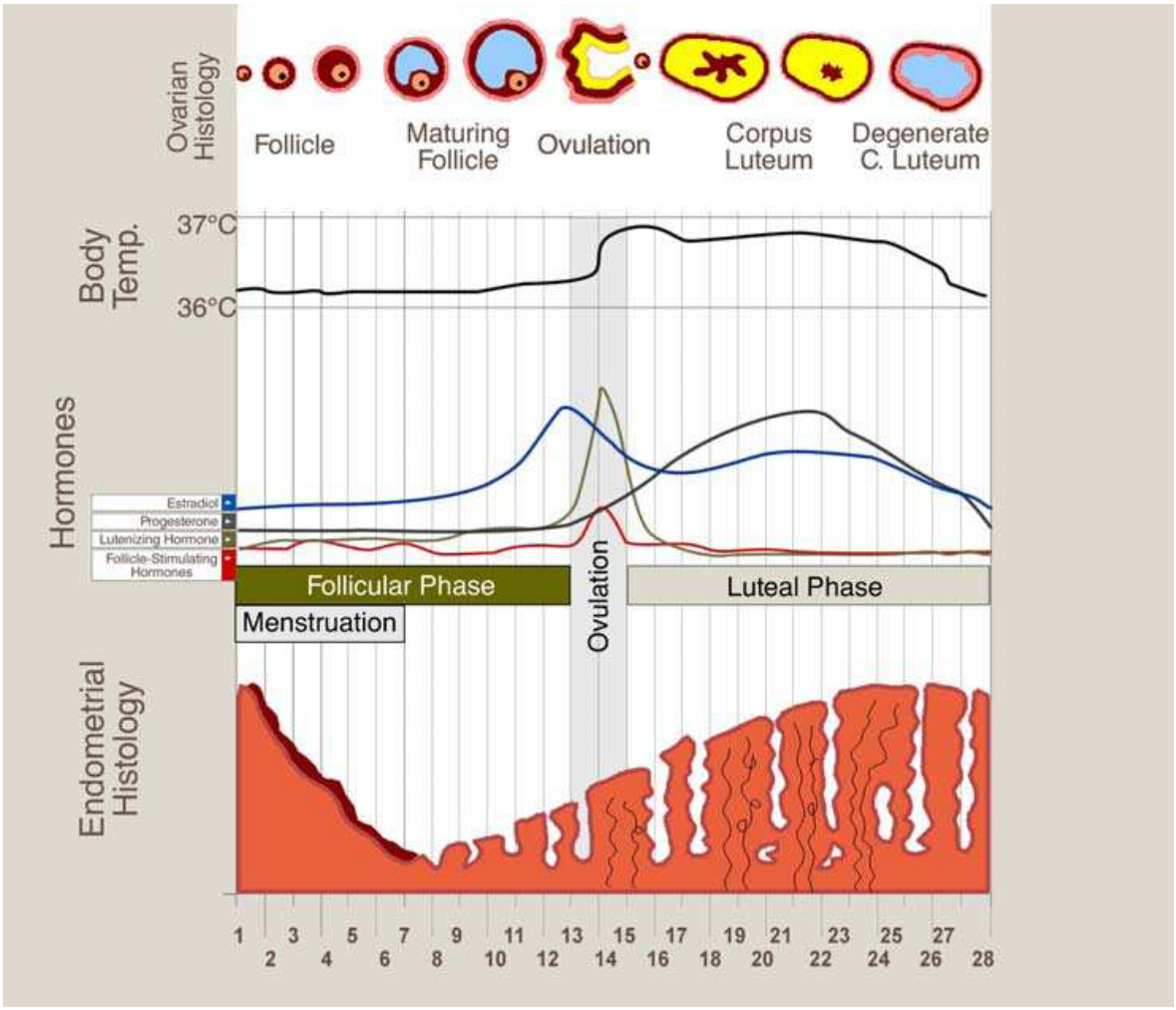


Fig 2.2: Menstrual cycle overview

2.3 Reproductive hormones

Fertility in women is tightly regulated by the hypothalamo-pituitary-ovarian (HPO) axis. Any derangement of the HPO axis results in menstrual irregularities and ovulation disorders, with consequent sub- or infertility (Kalro, 2003).

There are four major hormonal markers that characterise the menstrual cycle: two are of pituitary origin – LH and FSH – and two are of ovarian origin – oestradiol and progesterone (Ferin *et al;*1993). The hypothalamic-regulating hormones which orchestrate the activities of the anterior pituitary are luteinizing-releasing hormone (LHRH), corticotrophin-releasing hormone (CRH), growth hormone-releasing hormone (GHRH), somatostatin, thyrotrophic hormone-releasing hormone (TRH) and prolactin inhibiting factor (PIF or dopamine). LH-RH is a decapeptide, which is released in a pulsatile fashion to stimulate release of the gonadotrophins, FSH and LH, from the anterior pituitary (Willocks & Neilson, 1991).

The circulating levels of most major reproductive hormones have been shown to fluctuate, often quite dramatically. This accounts for the large variations shown by individual cycles, even among successive menstrual cycles in the same woman (Ferin *et al.*, 1993).

Clues to some hormonal problems may be pointed out by history taking and general inspection of the female patient. For example, the presence of hirsutism may indicate androgen excess.

Hirsutism in conjunction with obesity may be associated with polycystic ovarian syndrome or Cushing's syndrome. Irregular cycles in association with a low body weight may indicate hypogonadotrophic ovulation

problems, as seen in women with eating disorders. A history of hypo- or hyperthyroidism may suggest associated ovulatory problem. A history of galactorrhoea may indicate the presence of hyperprolatinaemia and even the presence of a prolactinoma (Brugh *et al.*, 2002).

2.3.1 Ovarian sex steroid hormone secretion

Ovarian follicles respond to pituitary gonadotrophin secretion by synthesising the principal ovarian hormones oestradiol and progesterone. Increasing levels of oestradiol feedback to the pituitary gland via a negative – feedback mechanism, resulting in decreased secretion of FSH and increased secretion of LH. This results in a marked increase in LH secretion, known as the LH surge, which triggers ovulation. With ovulation, the ovarian follicle is converted into a corpus luteum and begins secreting progesterone.

During a full reproductive cycle, one oocyte is brought to maturity before ovulation. In the process of bringing one oocyte to maturation, a number of oocytes are stimulated to partial maturation but subsequently undergo atresia before reaching ovulation. During the process of follicular maturation, pre-granulosa cells are stimulated by FSH to become granulosa cells, which begin secreting oestradiol. The binding of FSH to receptors in the granulosa cells causes granulosa cell proliferation, increased binding of FSH, and

increased production of oestradiol. The follicle with the greatest number of granulosa cells, FSH receptors, and the highest oestradiol production becomes the dominant follicle from which ovulation will occur (Beckmann *et al.*, 1995).

As a primordial follicle is stimulated, the *pretheca* cells surrounding the *granulosa* cells become theca cells. The *theca* cells secrete androgens which serve as the precursors for the oestradiol production by the granulosa cells (Beckmann *et al.*, 1995).

2.3.2 Hypothalamic GnRH secretion

Hypothalamic GnRH is secreted in a pulsating manner from the arcuate nucleus of the hypothalamus. GnRH secretion is influenced by oestradiol and catecholamine neurotransmitters. The neurotransmitters may help explain psychogenic influences on the reproductive cycle. GnRH reaches the anterior pituitary gland through the hypothalamic– pituitary portal plexus. Pituitary gonadotrophin secretion is stimulated and modulated by the pulsating secretion of GnRH. Surgical ablation of the arcuate nucleus in animals disrupts ovarian function, as does continuous infusion of GnRH agonists. Ovarian function can be restored by the pulsating infusion of GnRH at 70 – 90 minute intervals. (Beckmann *et al.*, 1995).

2.3.3 Prolactin

Prolactin is associated with the gonadotrophins, as well as with the growth hormone, and is produced by the mammotropes (lactotropes). Secretion is controlled by dopamine, which acts as a prolactostatin – inhibits prolactin secretion, serotonin, endorphin and thyroliberin, which stimulates prolactin secretion; and explains why stress (exercise, trauma, myocardial infarction), may promote secretion. Prolactin levels may be elevated in both hyper- and hypothyroidism, because thyroliberin secretion may be increased in both conditions (Meyer *et al.*, 1997).

Prolactin release is under a tonic inhibitory control by the hypothalamus. The secretion of prolactin is also influenced by (Ferin *et al.*, 1993):

- *Oestradiol*: The ovarian hormone oestradiol augments prolactin release. Oestradiol action is probably responsible for differences in prolactin concentrations in the adult versus the pre-pubertal or menopausal woman.
- *Sleep:* There is a moderate rise in prolactin concentrations during sleep.
- *Stress:* Several types of stress (e.g. cold, heat, physical aggression or surgery) are all known to increase prolactin release. Similarly, certain types of exercise will also result in increased prolactin levels.

• *Pharmacological agents:* Several drugs increase prolactin, mostly by decreasing dopamine activity through specific mechanisms. Tranquilizers may block dopaminergic receptors, such as the phenothiazine derivatives, or inhibit dopamine re-uptake from the interneuronal cleft, such as the tricyclic depressants.

• The suckling stimulus: The amount of prolactin released is proportional to the frequency of suckling; hence, levels of prolactin characterising the postpartum period are related to the amount of suckling.

• *GnRH*: In some superfused pituitary cell cultures, it was found that GnRH can stimulate prolactin release. This effect is not due to a direct action of the neurohormone on the lactotrope, but rather to the apparent production of a paracrine factor by the gonadotrope, which in turn stimulates the lactotrope to secrete prolactin. Prolactin is best known for the multiple effects it exerts on the mammary gland. However, it also exerts effects on other targets important to the reproduction of the mammalian species. The varied effects of prolactin on the mammary gland include growth (mammogenesis), synthesis of milk (lactogenesis), and maintenance of milk secretion (galactopoiesis). There are data suggesting that prolactin influences reproductive behaviour. In humans, high prolactin levels are associated with psychosomatic reactions including pseudopregnancy. There are prolactin-

receptors in the ventromedial nucleus of the hypothalamus, an area which controls female sexual behaviour (Freeman *et al.*, 2000).

Causes of hyperprolactinaemia include: the growth of a prolactin-producing adenoma; other tumours of the pituitary region which block the inhibitory influence of the hypothalamus; certain endocrine diseases, e.g. primary hypothyroidism; polycystic ovarian syndrome; antidepressants; antihypertensives and oestrogen.

Hyperprolactinaemia can disturb ovarian physiology at several levels, including follicular maturation and steroidogenesis, ovulation, the process of luteinization, and the corpus luteum function (Crosignani *et al.*, 1999). Increasing prolactin levels are frequently associated with disturbances of the menstrual cycle. Most commonly, these are seen in patients with a prolactin-producing pituitary adenoma. Thus, in the initial evaluation process of the infertile patient with irregular menses or amenorrhoea, it is always important to measure prolactin concentrations (Ferin *et al.*, 1993).

2.3.4 Thyroid-stimulating hormone(TSH)

TSH is released from the anterior pituitary in response to TRH, a tripeptide synthesised in the supraoptic and supraventricular nuclei (de Swiet *et al.*, 2002).

TSH contains 209 amino acids arranged in two polypeptide chains. Secretion is controlled by thyroliberin (TSH-RH) – the release is stimulated by stress, and the plasma level of thyroid hormone through negative feedback on the hypothalamus and particularly on the anterior pituitary. Thyrotropin controls the structure of the thyroid gland as well as each phase of its function (Meyer *et al.*, 1997).

TSH also has these effects on the thyroid: it increases its size, vascularity, iodine uptake, protein synthesis, storage of colloid and the secretion of T3 and T4 (de Swiet *et al.*, 2002).

Thyroid dysfunction is more common in women than in men. Clinical manifestations of thyroid disease can be subtle and insidious. Various reproductive disorders ranging from abnormal sexual development to menstrual irregularities and infertility has been associated with thyroid disorders. Hyper and hypothyroidism can result in menstrual irregularities and compromise fertility (Kalro, 2003).

Oligomenorrhoea seems to be the most common menstrual disorder in hyperthyroidism and may progress to amenorrhoea. Amenorrhoea is a feature of severe hyperthyroidism, with elevated LH and FSH levels, loss of midcycle LH peak, and consequent anovulation and low progesterone levels. Excess thyroid hormones typically increase sex hormone binding globulin

(SHBG) production and serum levels, reflecting increased tissue response to these hormones.

Circulating total oestrogen and testosterone levels are therefore increased, but active or free fractions are often reduced (Kalro, 2003).

Hypothyroidism often causes polymenorrhoea and oligomenorrhoea. It occasionally causes anovulation and rarely amenorrhoea. Occasionally, hypothyroidism may be associated with prolonged periods of amenorrhoea and anovulation. Patients with hypothyroidism have reduced levels of SHBG and consequently reduced levels of circulating oestrogens and testosterone. With anovulatory cycles, LH and FSH may also be reduced (Kalro, 2003).

Hypothyroid states are often associated with increased thyrotropin-releasing hormone levels, which increase both TSH and prolactin levels. Hyperprolactinaemia from longstanding primary hypothyroidism may be responsible for varying degrees of ovulatory dysfunction from luteal-phase insufficiency to oligomenorrhoea or amenorrhoea. (Kalro,2003).

2.3.5 Luteinizing hormone and follicle-stimulating hormone

The pituitary gonadotrophins FSH and LH are protein hormones secreted by the anterior pituitary gland (Beckmann *et al.*, 1995). LH and FSH are glycoproteins from the family which includes TSH and human chorionic gonadotrophin.

These hormones consist of a common ∝– subunit and a specific ß – subunit. Both are glycosylated, which determines their bioactivity and half- life (de Swiet *et al.*, 2002).

Secretion of the gonadotrophins, FSH and LH, is controlled by luliberin. This stimulates secretion of LH more effectively than follitropin secretion, the plasma levels of the sex hormones (oestradiol and progesterone in females) through positive and negative feedback. It is also controlled by inhibin, a hormone produced by the Graafian follicles in females.

Luliberin also inhibits the release of FSH (Meyer *et al.*, 1997). FSH and LH are also secreted in a pulsating fashion in concert with the pulsating release of GnRH. The magnitude of secretion and the rates of secretion of FSH and/or LH are determined by the levels of ovarian steroid hormones and other ovarian factors. When a woman is in a state of relative oestrogen deficiency, the principal gonadotrophin secreted is FSH. As the ovary responds to FSH secretion with oestradiol production, there is a negative feedback to the pituitary gland to inhibit FSH secretion and facilitate LH secretion (Beckmann *et al.*, 1995).

LH and FSH act on the gonads to stimulate gametogenesis and hormone synthesis.During the follicular phase, FSH and LH stimulate oestrogen

synthesis by the developing follicle. This initially feeds back to the level of the hypothalamus and possibly to the pituitary to inhibit FSH and LH release (de Swiet *et al.*, 2002).

FSH and LH have important actions on the ovary: the main effect of FSH is to stimulate growth and development of Graafian follicles, while LH acts to cause ovulation. Ovarian steroid hormones are produced through the actions of FSH and LH. As the Graafian follicle enlarges, increasing amounts of the oestrogen, oestradiol, are produced. With the mid-cycle surge of LH, ovulation occurs and the Graafian follicle is converted into a corpus luteum from which mainly progesterone is secreted (Willocks & Neilson, 1991).

A sophisticated system of feedback loops controls the sequence of co-ordination of endocrine events during the menstrual cycle. The increasing amounts of oestradiol produced by the Graafian follicle cause negative feedback to the hypothalamus, inhibiting release of LH-RH, and therefore also of FSH. As the levels of oestradiol continue to rise, however, a positive feedback loop is triggered to the anterior pituitary which produces a surge in FSH and, more importantly, a very large surge in LH to cause ovulation. As the amounts of oestradiol and progesterone produced by the fading corpus luteum decrease, a production of FSH picks up and the next cycle commences (Willocks & Neilson, 1991).

In regard to LH secretion, the most striking event is a spectacular and abrupt rise in concentrations at the end of the follicular phase: the pre-ovulatory surge.

Mean duration of the gonadotrophin surge is 48 hours. It is estimated that ovulation occurs about 18 hours after the LH peak, or 36 hours after the initiation of the LH surge (Ferin *et al.*, 1993).

FSH also rises at the end of the follicular phase as part of the pre-ovulatory gonadotrophin surge, but this increase is more modest than that for LH. Of importance to FSH secretion is the slight but physiologically very significant rise in FSH on the day(s) preceding or on the day of menstruation. Peak FSH values at this time are reached about 24 hours after menstrual flow has started: the early follicular phase FSH rises. This is the only time in the menstrual cycle at which the FSH:LH ratio favours FSH (Ferin *et al.*,1993).

Quantitative relationships between ovarian steroids and FSH release determine the amounts of FSH released at the end to the menstrual cycle. Sub-normal FSH release or abnormal FSH:LH ratios during the inter-menstrual period may result in deficient follicular growth, a delay in ovulation, and/or deficiencies in the secretory activity of the corpus luteum (presumably because of decreased amount of tissue available for luteinization), decreased progesterone secretion (the inadequate luteal phase

syndrome), and potential adverse effects on the implantation process (Ferin *et al.*, 1993).

2.3.6 Oestrogen and progesterone

Following ovulation, the corpus luteum continues to synthesise and release oestrogens and progesterone. Their production peaks 7 days after ovulation and thereafter declines unless conception and implantation occurs. Here the developing embryo releases human chorionic gonadotrophin (hCG) into the maternal circulation which maintains corpus luteum function (de Swiet *et al.*, 2002).

Progesterone is predominantly produced by the corpus luteum in the non-pregnant female. Small amounts are produced by the developing follicle and adrenals (Meyer *et al.*, 1997).

2.3.6.1 Oestrogen

Properties of oestrogen (de Swiet *et al.*, 2002):

- Structure: Stimulates endometrial growth, maintenance of vessel and skin, reduces bone resorption, increases bone formation, increases uterine growth.
- Protein synthesis: Increases hepatic synthesis of binding proteins.
- Coagulation: Increases circulating levels of factors II, VII, IX, X, antithrombin III and plasminogen, increases platelet adhesiveness.

- Lipid: Increases high-density lipoprotein (HDL) and reduces low-density lipoprotein (LDL), increases triglycerides, reduces ketone formation, increases fat deposition.
- Fluid balance: Salt and water retention.
- Gastro–intestinal: Reduces bowel motility, increases cholesterol in bile.

Although only minute amounts of oestrogen are secreted by the adrenal cortex, it is responsible for most of the oestrogens formed outside the ovaries. This is because it releases androstenedione and dehydroepiandrosterone (DHEA) which are converted to oestrogens by fat cells, hair follicles, etc. Apart from the small amounts produced by the adrenals, most oestrogens are synthesised by the cells of the corona radiata, theca interna and corpus luteum. Some oestrogen is formed from circulating testosterone. The ovary produces two oestrogens, namely, oestradiol and oestrone; the former is biologically more potent (Meyer *et al.*, 1997). Functions of oestrogens include the (Meyer *et al.*, 1997):

1. Promotion of follicle development and ovulation.
.
2. Stimulation of proliferation of the epithelial cells of the uterine tubes, uterus an vagina.
3. Stimulation of protein synthesis, e.g. contractile proteins in the myometrial muscle fibres of the uterus.

4. Reduction of the membrane potential of the myometrial muscle fibres thus increasing their sensitivity to oxytocin and prostaglandin.
5. Stimulation of duct growth in the mammary glands and involved in lactation.
6. Primary responsibility for the development of the female characteristics.
7. Involvement in skeletal growth and the maintenance of the structural integrity of bones.

2.3.6.2 Progesterone

Properties of progesterone (de Swiet *et al.,* 2002):

- Structure: Enhances endometrial receptivity, maintains myometrial quiescence, breast development.
- Respiration: Increases respiratory drive.
- Lipid: Reduces HDL and increases LDL.
- Fluid balance: Promotes sodium excretion.
- Bowel: Reduces bowel motility.
- Metabolism: Increases body temperature.

The most important function of progesterone is the regulation of endometrial

receptivity (de Swiet *et al*). Other functions of progesterone are that it (Meyer *et al.*, 1997):

1. Stimulates the secretory activity of the uterine tubes, uterus and vagina.
2. Is responsible for the pregestational changes in the endometrium, and together with oestrogen is responsible for the cyclic changes that occur in the cervix and the vagina.
3. Increases the membrane potential of the myometrial muscle fibres, thus decreasing their sensitivity and excitability to oxytocin and prostaglandin. This explains why progesterone therapy is sometimes so effective in threatening abortion.
4. Prevents ovulation when present in large amounts.
5. Decreases the number of oestrogen receptors in the endometrial muscle fibres.
6. Promotes protein anabolism.
7. Is responsible for a rise in body temperature at the time of ovulation.
8. Stimulates alveolar formation in the breasts during pregnancy.
9. Stimulates respiration.
10. Antagonises the action of aldosterone on the kidney.

Oestradiol and progesterone also act on the endometrium – the lining tissue of the uterine cavity. Oestradiol stimulates growth of all elements of the endometrium.

Under the influence of progesterone from the corpus luteum during the second half of the cycle, the endometrium is converted from a proliferative pattern to a secretory pattern, as the endometrial glands become tortuous and convoluted.

As progesterone and oestradiol levels fall towards the end of the cycle, the endometrium can no longer be sustained, so it breaks up and is cast off in the process of menstruation (Willocks & Neilson).

Oestradiol secretion remains low during the early follicular phase period, but increases 1week prior to the mid-cycle gonadotrophin surge ; first at a slow, then at a very rapid, quasi-exponential rate to reach a peak at the time of the onset of the LH surge: the late follicular phase oestradiol peak. Within a few hours after the initiation of the mid-cycle gonadotrophin surge, oestradiol concentrations fall abruptly. They rise again with the appearance of the corpus luteum. Progesterone secretion remains insignificant throughout the follicular phase, rises suddenly and modestly about 12 hours prior to the onset of the LH surge, then remains at a plateau for about 12 hours: the pre-ovulatory progesterone rise. Progesterone rises again 36 hours after the onset

of the LH surge.

During the luteal phase, levels of both progesterone and oestradiol rise, to reach a maximum about six to nine days after the mid-cycle gonadotrophin surge: the luteal phase oestradiol and progesterone secretory curve (Ferin *et al.*, 1993).

2.3.7 Testosterone

Nearly all circulating testosterone is bound to SHBG and albumin, with free testosterone being the most biologically active form. When elevated insulin levels are present, SHBG levels decrease while free testosterone levels increase (Hunter & Carek, 2003).

The greater the body mass index, the higher the testosterone levels, and therefore hirsutism is more common in overweight anovulatory women (Speroff *et al.*, 1999).

Hirsutism is defined as the presence of excessive terminal hair in androgendependent areas of a woman's body (Hunter & Carek, 2003). Alopecia and acne are also consequences of hyperandrogenism (Speroff *et al.*, 1999).

2.4 Infertility

Infertility is defined as one year of attempted conception without success (Smith *et al.*, 2003).

2.4.1 A concept of infertility

The desire to reproduce is an intensely motivating human force. Because of its personal nature, couples may also experience strong religious, cultural and societal pressures to conceive. Therefore, it is understandable that people experiencing infertility often perceive it as a serious life crisis. Societal and parental pressures for propagation of the family name can place a psychological burden on the infertile couple. This central role of reproduction in the human experience has contributed greatly to the desire of couples to overcome infertility. Childbearing is an important aspect of most marriages. For most couples, the conception and raising of children are the expected outcomes of their sexual relationship. To some extent, it has led to the rapid evolution of technologic advances in reproductive biology. The physical, psychological and financial challenges of assisted reproductive technology may further impact the couple (Monga *et al.*, 2004; Seibel). Fertility in both men and women is at its maximum in the mid-twenties and, in women, declines after the age of 30 years (Willocks & Neilson, 1991).

The fertility of a marriage is a sum of the fertilities of the two partners. Low fertility in one can to some extent be balanced by high fertility in the other, whereas low fertility in both partners may result in infertility. This explains why some couples fail to reproduce, yet when they separate and each takes a new mate, they both proceed to have children (Tindall, 1987).

Primary infertility is the term used to describe those couples who have never achieved a pregnancy, whereas secondary infertility defines patients who have previously achieved a pregnancy of any type and duration (DeCherney, 1990).

2.4.2 Causes of infertility

The number of infertile couples seems to be increasing because many couples postponed the start of a family. Delaying pregnancy decreases the number and quality of available eggs and allows a greater length of time for women to develop unwanted sequelae of conditions such as endometriosis, uterine fibroids, and pelvic inflammatory disease (PID) (Brugh *et al.*, 2002).

In sub-Saharan Africa sexually transmitted diseases (STDs) most often implicated in infertility are gonorrhoea, chlamydia and syphilis. These STD's either prevent conception by scarring the Fallopian tubes as a result of PID, or in the case of syphilis, by causing foetal loss through spontaneous

abortion or stillbirth. Although there are causes of infertility in addition to STDs, epidemiologists agree that it is the transmission of STDs and the lack of treatment for these diseases that explains infertility in sub-Saharan women (Ericksen&Brunette, 1996).

In any series of infertile marriages, the main aetiological factor is found in the female more often than in the male (Tindall, 1989). In a retrospective study undertaken by Poppe *et al.* (2002), female origin was diagnosed in 45% of the couples with specific causes including endometriosis (11%), tubal disease (30%), and ovarian dysfunction (59%). Other variables contributing to infertility include age, history of sexually transmitted diseases, education, income level and parity (Green *et al.*, 2001).

Cigarette smoking has been demonstrated in multiple studies to impair fertility potential in a dose-dependent manner in both men and women. Smoking has adverse effects on tubal function, hormonal secretion and cervical mucus production. An association between high alcohol intake and an increased risk of infertility has been found. Alcohol ingestion has also been shown to cause a decrease in gonadotrophin levels and irregularities in ovulation (Brugh *et al.*, 2002; Eggert *et al.*, 2004).

Clinical syndromes in females which may be associated with infertility include (Priest,1985):

- Tubal spasm and hypogonadism
- Spontaneous abortion
- Hyperemesis and psychological vomiting
- Pre-eclamptic toxaemia
- Amenorrhoea and anovulation
- Anorexia nervosa

2.4.3 Infertility in Africa

The prevalence of infertility across sub-Saharan Africa has received scant attention in population research despite the well-known linkage between infertility, sexually transmitted diseases (STDs) and other reproductive tract infections. There is also mounting epidemiologic evidence that African women have the highest rates of disease induced infertility in the world (Ericksen & Brunette, 1996).

2.4.3.1 Infertility in black African women

The problem of infertility in sub-Saharan Africa has received little attention from researchers. It is obscured by the region's high fertility rates, which give rise to a global climate of concern over population growth and high fertility that is not conducive to the perception of infertility as a real problem (Hollos, 2003).

The incidence of infertility is estimated to vary from 10 – 20%, but it appears to be rising.Currently there is an increasing awareness of infertility in Africa as a serious social and public health problem. This increase in public awareness, as well as the availability and scope of infertility services, might be the contributing factor to the apparent increase in prevalence (Chigumadzi *et al*., 1998; Hollos, 2003).

In a continent where marriage is almost universal, and the purpose of marriage is children, infertility is often viewed as a major tragedy (Dyer, 2002). Loss of self-esteem, anxiety and depression, hopelessness, guilt and marital difficulties are all recognised consequences of infertility. As the desire to have children has been said to be amongst the strongest emotions that people experience, it is not surprising that infertility has been considered life's worst experience by those who suffer from it (Dyer *et al*., 2002a). Infertility is recognised as a major cause of divorce and spousal abandonment throughout the continent. (Dyer *et al.,* 2002a). In Nigeria, fertility from the men's point of view means that additional offspring adds to the power and prestige of their sub- lineage or family.Prestige is determined by the number of adult male followers a man can have.

Economically in Nigeria, children are important in establishing claims to landholdings in the community, in competition with other sub- lineages, as

with many other cultures in Africa (Hollos, 2003).

Women in Africa are particularly affected by their infertile status. Their social status and security usually depends directly on fertility. Those who cannot reproduce are at a substantial risk of divorce, stigmatization, socio-economic deprivation and abuse (Dyer, 2002). For example, infertile women in Mozambique are excluded from important social events and ceremonies. In Gambia, childless women have very few rights to inherit property from their husbands (Dyer *et al.*, 2002a). With children, a woman's prestige and value is assured and increased with each additional child. A woman is considered to be an unfortunate being if she is infertile. Not only is she thought to be disadvantage economically, but her childlessness also prevents her from attaining full adult womanhood (Hollos, 2003). In a study carried out at a hospital in Durban, South Africa, women claimed to frequently suffer abuse from their husband's family, and the level of abuse was deemed enough to push a woman to suicide (Dyer *et al.*, 2002b).

It is a fair assumption that the cost of assisted reproduction in most African countries is not covered by public health care or private insurance companies. In this context, cost becomes a critical factor, and any further increase may further limit access to treatment.

This is in line with the findings of a study carried out in the United States,

where it was found that users of infertility services were more likely than non-users to be white and to have higher incomes. This further complicates the matter of infertile black women seeking treatment, due to the costs which they may not be able to afford (Dyer, 2002; Green *et al.*, 2001).

2.5 Anovulation

The causes of anovulation can be divided into two categories. One category occurs as a result of hypothalamic-pituitary dysfunction. Hypothalamic-pituitary dysfunction resulting in hypogonadotrophic hypogonadism is characterised by a selective failure of the pituitary gland to produce LH and FSH. In addition to this, women with a low body mass index (BMI) [weight (kg)/ height (m^2)] (for example < 20kg/m^2), may develop amenorrhoea because of a physiological reduction in the hypothalamic production of gonadotrophin-releasing hormone (Hamilton-Fairley & Taylor, 2003).

The second category of anovulation has ovarian causes. In this category, polycystic ovarian syndrome is the most common cause of anovulatory infertility. The primary abnormality is the excess androgen production within the ovary that leads to the recruitment of large numbers of small pre-ovulatory follicles. The androgen fails to respond to normal concentrations of FSH (Hamilton-Fairley & Taylor, 2003).

Ovulation can be affected by means of a multitude of factors. The three most common ones are (Seibell, 1997):

(1) Excessive weight loss or weight gain;

(2) excessive exercise; and

(3) extreme emotional stress.

Twenty per cent above or below ideal body weight may affect ovulation. Also, it must be remembered that obese people may be protein deficient and thin people may be eating quite well. The relationship between excess body fat and ovulatory disturbances appears stronger for early-onset obesity (Seibell, 1997).

Obese women develop menstrual disorders which are more often anovulation than amenorrhoea. Obese women have an excess number of fat cells in which extraglandular aromatization of androgens to oestrogens occurs. They also have lower circulating levels of SHBG, which allows a larger proportion of free androgens to be converted to oestrone.

The decrease in SHBG allows an increase in free androgen levels, which initially are removed by an increased rate of metabolic clearance. This compensatory mechanism diminishes over time, and hirsutism can develop (Scherzer & McClamrock, 1996).

As a woman ages, the ovarian follicles diminish in number and become less sensitive to FSH. The process of ovulation becomes increasingly inefficient, less regular, and less predictable than in earlier years. A woman will begin to notice changes in her reproductive cycle at around age 38 to 42. Initially, she will notice a shortening of the cycle length. With increasing inefficiency of the reproductive cycle, the follicular phase shortens, but the luteal phase is maintained at normal length. With the passing of time, some cycles become anovulatory, so that the frequency of ovulation decreases (Seibell,1997). Another common cause of anovulation in women is hyperprolactinaemia. It is caused by a pituitary micro-adenoma. This leads to a reduction in the production of pituitary luiteinizing hormone and FSH (Scherzer & McClamrock, 1996; Hamilton-Fairley & Taylor, 2003).

2.6 Amenorrhoea

Recent observations have clearly demonstrated that, while the hypothalamic pituitary ovarian axis is capable of maintaining ovulatory cyclicity on its own, multiple endogenous or environmental influences may impinge on the normal activity of the pulse generator, usually to decrease GnRH pulse frequency and thereby induce cyclic dysfunction. These conditions are usually diagnosed as hypothalamic amenorrhoea.

Frequent causes of hypothalamic amenorrhoea are related to exercise, diet or stress (Ferin *et al.*, 1993).

Hypothalamic amenorrhoea is characterised by amenorrhoea, Hypo-oestrogenism, low or normal serum gonadotrophins, and a broad spectrum of abnormal patterns of hypothalamic GnRH secretion (Perkins *et al*., 2001).

Evidence indicates that psychological, metabolic, and/or nutritional stress could be the basis of hypothalamic amenorrhoea (Andrico *et al.,* 2002).

2.7 Overweight and obesity

An early reference to the influence of obesity on menstruation can be found in the writings of Hippocrates. In an essay on the Scythians, which appears under the heading the influence of climate, water supply and situation on health', their reproductive function is described in the following terms: "The girls get amazingly flabby and podgy… People of such constitution cannot be prolific… fatness and flabbiness are to blame. The womb is unable to receive the semen and they menstruate infrequently and little". The 'control subjects' are the serving wenches: "As good proof of the sort of physical characteristics that are favourable to conception, consider the case of serving wenches. No sooner do they have intercourse with a man than they become

pregnant, on account of their sturdy physique and their leanness of flesh" (Franks *et al.*, 1996).

Obesity in women is associated with a number of negative health consequences. In the western world, a body mass index (BMI) > 25 kg/m^2 has been associated with poor education, low income and low socio-economic status. An increased prevalence of metabolic diseases such as diabetes mellitus, gall bladder disease, atherosclerosis, myocardial infarction and stroke, has also been observed in obese individuals, as well as negative psycho-social attitudes from other persons (Wass *et al.*, 1997). Recently, visceral fat accumulation, rather than the total amount of subcutaneous fat, has been shown to be associated with impaired glucose tolerance and lipid metabolism. Upper body obesity (or android obesity), has been identified as an independent risk factor for stroke, CHD and death in both men and women (Kruger, 1999).

2.7.1 Impact of obesity on fertility

Obesity produces a variety of alterations in the reproductive system in humans (Lashen *et al.*, 1999). Obesity affects ovulation, the response to fertility treatment, pregnancy rates and outcome. The fertility of obese women is lower than that of normal weight women in natural cycles and in-vitro fertilisation programmes (Clark *et al.*, 1995; Clark *et al.*,1998).

According to Reid & van Vugt (1987), one of the first attempts to document the relationship between obesity and menstrual dysfunction was presented in 1952.

Researchers found a higher incidence of obesity in women with amenorrhoea than they did in the population of normal cycling control subjects with normal cycles. Lashen *et al.*1999) reported on a study where obese women with amenorrhoea resumed their menses with weight reduction alone. Most cases of amenorrhoea in women develop shortly after a period of weight gain (Reid & van Vugt, 1987).

Today a BMI of >27 kg/m² is considered to be a factor of decreased fertility. The ovaries of obese women have increased capsular hyalinisation, and increased numbers of atypical or atretic follicles (Crosignani, 2002; Glass *et al.*, 1981).

The distribution of body fat is clearly related to infertility. The adverse health implications of obesity are significant. This is evidenced in the many associations with different reproductive hormones, as well as its effect on the menstrual cycle. Therefore, all obese patients, regardless of their menstrual function or fertility, should be encouraged to participate in some form of structured weight control programme (Reid & van Vugt, 1987).

2.7.2 Endocrine abnormalities

As previously stated, obesity affects the results of fertility therapy (Maelli & Grazi, 2002). Obesity is associated with hormonal disturbances, decreased sex hormone binding globulin, elevated serum oestradiol and elevated levels of androgens (Chong *et al.*, 1986).

Obese anovulatory women show higher concentrations of oestrone and/or free oestrone than do either ovulatory obese women or women with normal weight.The fact that adipose tissue can act as a steroid reservoir and a site of peripheral conversion of androgens to oestrogen, could account for the greater oestrogen concentrations in obese women than in women of normal weight.

However, this does not explain the differences in circulating oestrogen concentrations between weight-matched anovulatory and ovulatory obese women. Weight loss is not accompanied by a fall in serum oestrone concentrations, as one might expect with a reduction in adipose tissue. Mobilisation of steroids from the sizeable fat tissue reservoir could be one explanation (Reid & van Vugt, 1987).

Oestrogen augments the release of LH and inhibits the release of FSH, thus leading to an increased LH/FSH ratio. The elevated LH level in turn stimulates androgen secretion by *theca* cells of the ovary, providing the

precursors for continued oestrogen production in adipose tissue. This vicious cycle results in simultaneous occurrence of hyperandrogenism and hyperoestrogenism. Long term acyclic oestrogen exposure may lead to excessive endometrial growth, resulting initially in oligomenorrhoea interspersed with episodes of menorrhagia. In some women this ultimately leads to the development of endometrial hyperplasia or adenocarcinoma (Reid & van Vugt, 1987).

Basal serum LH and FSH are normal in obesity, but nocturnal LH secretion is decreased. Serum FSH or serum LH might be elevated in obese women. In subjects with gonadal dysgenesis, there is an inverse correlation between 24-hour intergrated serum LH levels and total body water to body weight, a ratio that is inversely related to the percentage of body fat. The pre-ovulatory serum FSH rise is sub-normal in obese pre-pubertal girls than in girls of normal weight. Data suggest that amenorrhoea in obesity is not due to primary ovarian failure, which should be associated with elevated serum LH and FSH, but rather to some hypothalamic-pituitary abnormality (Glass *et al.*, 1981)

.

Hyperandrogenism may be aetiologically related to amenorrhoea in obesity. Amenorrhoeic subjects have elevated free androgen levels, while obese eumenorrhoeic subjects do not. Therefore, hyperandrogenism is associated

with the amenorrhoea of obesity. The hyperandrogenism is not secondary to the amenorrhoea, because amenorrhoeic subjects of normal weight do not have elevated free androgen levels. The conversion of androstenedione to oestrone is increased in obese women. This enhanced conversion of androgens to oestrogens may be carried out in the adipose tissue itself, since fat in vitro can convert androstenedione to oestrone and testosterone to oestradiol.

Obese women often have menstrual cycles with inadequate progesterone production during the luteal phase; a change that may account for decreased fertility (Glass *et al.*,1981).

2.7.3 Obesity and spontaneous ovulation

Obesity is associated with three alterations that interfere with normal ovulation, and weight loss improves all three. They are (Speroff *et al.*, 1999):

(1) increased peripheral aromatisation of androgens to oestrogens;

(2) decreased levels of SHBG resulting in increased levels of free oestradiol and testosterone ; and

(3) increased insulin levels that can stimulate ovarian stromal tissue production of androgens.

Excessive visceral body fat is associated with insulin resistance,

hyperinsulinaemia and high insulin-like growth factor – I (IGF – I) bioactivity as a result of a decreased concentration of insulin- like growth factor binding protein -1 (IGFBP – 1). IGF – I is a sensitizing factor that enhances the ability of granulosa cells in small antral follicles to respond to FSH facilitating the induction of LH receptors. In the thecal cells, both insulin and IGF-I stimulate ovarian androgen synthesis. Therefore, insulin and IGFs are important intra-ovarian regulators, and systemic or local disturbances may result in alterations of spontaneous ovulation (Galtier-Dereure *et al.*, 1997).

In addition to this direct role on ovarian function, body fat appears to be strongly related to the activity of the hypothalamo-pituitary axis. Excessive weight particularly influences the concentration of LH, which is probably the key hormone in the relationship between reproduction and metabolism. Obesity is associated with excessive LH concentrations, and it has been shown that a high concentration of LH results in a lower chance of conception (Galtier-Dereure *et al.*, 1997).

The mechanisms by which nutritional status influences hypothalamic activity are poorly understood. The aromatizing function of adipose tissue is possibly a means by which obesity impairs gonadotrophin secretion. As hyperinsulinaemia decreases SHBG concentrations, obesity is associated

with high concentrations of unbound androgens.

Excessive bioavailability and aromatising of androgens generates increased oestrone concentrations, which in turn triggers a rise in LH secretion. LH subsequently stimulates the production of ovarian androgens, thus enhancing substrate availability for the aromatising system (Galtier-Dereure *et al.*, 1997).

2.8 Polycystic Ovarian Syndrome (PCOS)

The basic pathophysiologic defect in polycystic ovarian syndrome (PCOS) is not known (Guzick, 2004). The consequences of PCOS include infertility, diabetes, abnormal uterine bleeding, hirsustism, alopecia, acne, increased risk of endometrial cancer and cardiovascular disease.

2.8.1 Definition

According to Slowey (2001), the definition of PCOS has three requirements. First is the presence of an ovulatory disorder. This may range from oligo-ovulation to anovulation with amenorrhoea. The second feature of PCOS is evidence of androgen excess, either clinical or on laboratory testing. Finally, other sources of anovulation and hyperandrogenism must be ruled out. These sources include hyperprolactinaemia, hypothyroidism, late-onset congenital adrenal hyperplasia, Cushing's syndrome, or an androgen-secreting tumour.

In the United States, women are defined to have polycystic ovarian syndrome if they have chronic anovulation and evidence of androgen excess (Guzick, 2004).

Women with PCOS are seen primarily for menstrual irregularity, androgen excess, and infertility. Women with chronic anovulation and hyperandrogenism have been observed to have an increased prevalence of diabetes and increased risk factors for CHD. Many women with PCOS are similar to those with metabolic cardiovascular syndrome(i.e.Syndrome X), a CHD-associated clustering within the same individual of hyperinsulinaemia, glucose intolerance, dyslipidaemia and hypertension. The chronic anovulation of polycystic ovarian syndrome implies unopposed oestrogen and, therefore, an increased risk of endometrial cancer (Guzick, 2004).

2.8.2 Pathophysiology

Although the fundamental pathophysiologic defect in PCOS is unknown, these women have several interrelated characteristics, including insulin resistance, hyperandrogenism, and altered gonadotrophin dynamics. It is well known that obesity is associated with insulin resistance. Because women with PCOS are often obese, it is not surprising that they would have some element of insulin resistance. The extent of insulin resistance among

women with polycystic ovarian syndrome cannot be explained entirely by obesity (Guzick, 2004).

There is a strong correlation between insulin resistance and hyperandrogenism. Evidence would suggest that the direction of causation is from insulin to androgen, and not the reverse. Weight loss and insulin sensitisers, which also lead to a reduction in insulin, similarly are associated with a reduction in androgens, particularly testosterone and androstenedione (Guzick, 2004).

Another key pathophysiologic feature of PCOS is altered gonadotrophin-releasing hormone dynamics. Both lean and obese women with polycystic ovarian syndrome have increased LH pulse frequency and amplitude. Because androgen production by *theca* cells is LH dependent, it would seem to follow that the elevated levels of LH seen in women with PCOS are responsible for the excess androgen production (Guzick, 2004).

Although increased androgen production in women with PCOS is augmented by increased LH and is associated with anovulation, it can be argued that the proximate cause of the anovulation may be insufficient FSH. Follicles in the ovaries of women with PCOS do not mature fully, and the granulosa cells in these arrested follicles are low in number and in aromatase

activity. Therefore, oestradiol production by these follicles is limited (Guzick, 2004).

Absolute concentrations of FSH above a specified threshold are essential for both the initiation of pre-ovulatory follicle development, as well as the selection of a single preovulatory follicle. In the case of PCOS, the concentration of FSH may simply not rise above levels seen in the mid-follicular range of the normal menstrual cycle, which are insufficient to stimulate pre-ovulatory follicle development (Guzick, 2004).

2.9 Underweight

Underweight is a far less prevalent problem than overweight. Whether the underweight person needs to gain weight is a question of health and, like weight loss, it is highly an individual matter. People who are healthy at their present weight do not need to loose any weight. Those who are thin because of malnourishment or illness, however, might benefit from a diet that supports weight gain (Whitney & Rolfes, 2002)
.

Changes in the attitudes and practices of western society have led to a striking increase in the prevalence of this problem over the past decade. Widespread promotion of health and fitness has led to the participation in

sporting activities by unprecedented numbers of women at all competitive levels (Borini & Asch, 1993).

2.9.1 Low body weight

Low body weight can be caused by an intake insufficient in quantity to meet activity needs; excessive activity, such as in the case of compulsive athletes in training; poor absorption and utilisation of food consumed; a wasting disease, such as cancer or hyperthyroidism, that increases the metabolic rate and energy needs, and psychological or emotional stress (Laquatra, 2004). Aversion to fatness is not unique to athletes: western society has become preoccupied with the concept that thinness and success go hand-in-hand. Current fashions and television marketing lend the impression that the thin woman is more likely to succeed in all walks of life, ranging from finding a mate to entering the professional or business world. Not surprisingly, there has been a striking increase in the incidence of hypothalamic amenorrhoea and anorexia nervosa in today's women. The factors influencing menstrual function in these circumstances may be complex and are rarely the sole consequence of changes in body weight. Rather, current evidence suggests that a combination of physical, psychological, and nutritional stresses may act additively in the determination of the timing of the onset and rate of

pubertal progression, menstrual function, and fertility (Reid & van Vugt). The mechanism of the adverse influence of undernutrition on ovarian function is reasonably well understood. This is best illustrated, in women, by the phenomenon of weight loss-related amenorrhoea, in which there is a characteristic disturbance of the hypothalamic control of gonadotrophins by GnRH. In most cases there is a cessation in the release of the gonadotrophins and this is accompanied by decreased pituitary stores of the hormone (Franks *et al.*, 1996; Ferin *et al.*, 1993). Marked weight loss with physical or psychological stress will disrupt hypothalamic growth hormone (GnRH) production, leading initially to subtle endocrine abnormalities, such as luteal phase defects, and ultimately, to oligoamenorrhoea or amenorrhoea. The degree of hypothalamic dysfunction in such circumstances appears to be, in part, linked to the percentage of body fat rather than body weight. Alongside the hypothalamic disturbances, the serum oestradiol levels also fall and there is a reduction in plasma prolactin, together with loss of the nocturnal prolactin rise (Reid & van Vugt 1987).

2.9.2 Anorexia nervosa and bulimia nervosa

Functional disorders of the hypothalamus are the most frequently encountered hypothalamic disorders in clinical practice. The most extreme weight-related functional disorder of the hypothalamus is anorexia nervosa.

This disorder occurs primarily among adolescent white females from middle to upper social class backgrounds, and occurs more frequently in women than in men (Bates, 1992).

Anorexia nervosa is a disease characterised by refusal to maintain a minimally normal body weight, intense fear of gaining weight, body image distortion and amenorrhoea in postmenarcheal females. It is a condition characterised by voluntary self-starvation and emaciation. Weight loss is viewed as a sign of extraordinary achievement and self discipline (Schebendach & Reichert-Anderson, 2004).

Bulimia nervosa, on the other hand, is characterised by repeated episodes of binge eating followed by inappropriate compensatory methods such as purging, including self-induced vomiting or misuse of laxatives, diuretics, or enemas; or nonpurging, including fasting or engaging in excessive exercise (Schebendach & Reichert-Anderson, 2004).

Anorexia nervosa profoundly affects neuroendocrine function. The 24-hour secretion of gonadotrophins reverts to a pubertal pattern in anorectic women, and does not return to an adult pattern until weight is returned to normal. Sex steroid hormone secretion, associated with decreased gonadotrophin secretion, reverts to a pre-pubertal state, thus rendering these women child-like from the viewpoint of reproductive physiology. In a study reported by

Bates (1992), in women weighing between 53% and 64% of ideal body weight (IBW), the LH response was impaired, while the FSH response was equal to that observed in normally menstruating women. In the weight range of 79% to 88% of IBW, the LH response improved but remained less than that in normal women.

In women who regained to within 90% – 94% of IBW, the LH response was greater than or equal to that found in normal women (Bates, 1992).

Weight loss of approximately 10% of body weight in 1 year can cause amenorrhoea, regardless of premorbid weight. The association of amenorrhoea with anorexia nervosa is well established. Menstrual disturbances often begin prior to significant weight loss and persist after recovery to normal weight (Perkins *et al.*, 2001).

In patients with bulimia nervosa, menstrual irregularities are high. One might predict difficulties achieving pregnancy for women with bulimia nervosa on the basis of lower levels of LH or smaller spikes in LH levels. Abnormal LH secretion may be related to current weight that is less than 85% of the highest life time weight, rather than current low body weight (Crow *et al.*, 2002).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY AREA

Zaria is located at longitude 4.2667 and latitude 11.25 . The traditional city has an estimated population of 170,000 people (personal communication with department of health Zaria LGA) The major ethnic groups are Hausa and Fulani. Others are Yoruba, Igbo, Ebira, Idoma, Igala, and Tiv.

The Hausa and Fulani are two distinct tribes with similar cultural-religious practices. Inter marriage between these tribes occurs quite frequently especially in urban areas of Northern Nigeria. In places like Zaria it is difficult to come across an individuals who can claim to be a pure breed of any of the two major ethnic groups. The term Hausa-Fulani has therefore been unofficially adopted.The major occupation of Zaria people include farming, civil service and trading.

Social amenities available in Zaria includes primary schools, post primary institutions, tertiary institutions, primary health care centres, secondary health care centres and tertiary health care centres. Kaduna state water board supplies Zaria with water. The people of Zaria enjoy epileptic power supply from Power Holding Company of Nigeria (PHCN). There is an accessible

tarred road which links Zaria to major towns like Jos , Kaduna, Funtua, Sokoto and Katsina.

3.2 SAMPLE SIZE DETERMINATION

The sample size for the study was determined from a standard formula for the calculation of minimum sample size (Oyejide, 1992; Singha, 1996)

$$n = \frac{(Z\text{-}a)^2 (p) (1\text{-}p)}{(d)^2}$$

Where; n = minimum sample size; Z-a = the value of standard normal deviation which at 95%confidence level has been found to be 1.96; p = the best estimate of the population prevalence obtained from the above literature review. d = the difference between the true population rate and sample that can be tolerated, that is the absolute precision required(in percentage point) on either side of the population. In this case d is taken to be =0.05.

Ory (1990) reported a prevalence of 10-25% endocrine cause of female infertility in Africa.

Therefore using 10%, as the prevalence (p) the minimum sample size n was calculated as follows.

$$n = \frac{(1.96)^2 \ (0.1) \ (1-0.1)}{(0.05)^2}$$

$$= \frac{3.8 \times 0.1 \times 0.9}{0.003} = 114$$

The calculated minimum sample size was 114 patients.

10% attrition rate was added to account for fallout.

Which implies 114x10/100 = 11.4
=114 + 11.4 =125.4 patients.

Therefore, 125 infertile women would be recruited for the study.

Due to lack of client's compliance among the control groups 120 infertile women and 60 fertile women (controls) were recruited for the study.

3.3 INFORMED *CONSENT*.

Fully informed consent for inclusion into the study was obtained from each patient and control.

3.4 ETHICAL CONSIDERATIONS

Ethical approval was obtained from ethical and scientific committee of the ABUTH, Zaria in accordance with the declaration of Helsniki.

This is code of ethics on Human experimentation drafted by the World

Medical Association in Helsniki in 1964, as updated by the General Assembly in Tokyo 2004.

3.5 STUDY POPULATION:

The study was conducted at Ahmadu Bello University Teaching Hospital, Zaria, Northern Nigeria. A total of 180 female subjects were recruited for this study. This consisted of 120 infertile women and 60 apparently healthy fertile women of reproductive age as (control).

The target population were adult females who were in their reproductive age of 19-45yr attending infertility clinic of ABUTH, Zaria and apparently healthy fertile subjects (control group) from the population of staff and students of ABUTH, Zaria who were married and having children.

3.6 INCLUSION CRITERIA:

Patients aged between 16 and 45yr of age presenting to the above mentioned clinics with complaints of inability to conceive after one year despite regular and unprotected sexual intercourse were included in the study. Apparently healthy subjects who were married and having children were included in the study.

3.7 EXCLUSION CRITERIA:

Subjects who were below 16 but not married and above 45yr were excluded from this study. All infertile women who were currently on hormonal therapy for the treatment of their infertility were excluded.

All subjects who declined to give consent for inclusion were also excluded from the study. All pregnant and lactating women were excluded from the study. All control groups who were using hormonal injectable or oral contraceptives were excluded from the study. All control groups whose years of last child birth were more than five years were excluded.

3.8 Operational definitions

The following operational definitions are defined according to their uses in this study.

3.8.1 Anthropometric measurements

Anthropometry is the measurement of body size, weight, and proportions. Anthropometric measures can be used to evaluate nutritional status, whether it be obesity caused by over- nutrition, or emaciation resulting from protein-energy malnutrition (Lee & Nieman, 2003). Anthropometric measurements in this study include the measurement of the weight, height and body mass index (BMI).

3.8.2 Body mass index

Body mass index (BMI) is derived mathematically from the height and weight measures.

BMI = weight (kg) / Height (m^2)

BMI values correlate significantly with body fatness and obesity, and experts use them to help evaluate a person's health risks associated with underweight (Whitney *et al.*, 2002).

BMI can be categorized as follows according to the WHO (Laquatra, 2004):

Underweight < 18.5 kg/m^2

Normal 18.5 – 24.9 kg/m^2

Overweight 25.0 – 29.9 kg/m^2

Obese class I(mild) 30.0 – 34.9 kg/m^2

Obese class II (moderate) 35.0 – 39.9 kg/m^2

Obese class III (severe) > 40 kg/m^2

3.8.3 Biochemical parameters

The following biochemical measurements were taken, and the following cut-off points are given for women with normal menstruation (Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital, Zaria).

1.Serum progesterone refers to fasting serum progesterone concentration.

Serum progesterone concentration values for a normal menstruating female

is 3.0- 32.o ng/ml.

2. Serum LH refers to the serum LH concentration. During normal menstruation the reference range for LH is : 2.1 – 24.5mIU/ml.

3. Serum FSH refers to the concentration of serum FSH. Serum FSH concentrations for normally menstruating women : 3.6 – 36.2mIU/ml.

4. Serum prolactin refers to the serum prolactin concentration. Serum Prolactin concentrations for non-pregnant women: 8.4-22.0ng/ml.

5.Serum oestradiol refers to the serum oestradiol concentration. During normal menstruation the reference range is: 10- 907pg/ml.

3.9. SAMPLING TECHNIQUES

At the location of the infertility clinic of ABUTH, Zaria arrangements were made with the consultant in-charge where by consecutive subjects who satisfied the study inclusion criteria were selected.

A full medical history was obtained from the selected subjects using a self administered questionnaires. This was followed by clinical examinations, anthropometric measurements and collection of blood specimens. All findings were documented using a standard format.

3.9.1 ANTHROPOMETRIC MEASUREMENTS

All measurements were taken using standardised equipment.

3.9.1.1 **EQUIPMENT**

1. STADIOMETER

Height was measured using a stadiometer to the nearest 0.1cm. A stadiometer is a measuring stick attached to a vertical metal board with a movable horizontal head board. The stadiometer can measure up to two meters (Heymfield, 2002).

2 **WEIGHING SCALE:**

A platform weighing scale was used to measure the subjects weight to the nearest 0.1kg.(Lee and Nieman,2003).

3.9.1.2 MEASURING PROCEDURES

Anthropometric measurements were carried out according to Standardised methods and techniques (Lee and Nieman, 2003).

WEIGHT

The weighing scale was placed on a hard surface to ensure accurate reading. The scale was adjusted to zero before the subject stands on it, wearing minimal clothing and no shoes. The subject was instructed to stand still with

weight evenly distributed on both feet while the reading was taken to the nearest 0.1kg.

HEIGHT

Height was measured with the subject standing erect with weight equally distributed on both feet with heels together and flat on the floor. The subject looked straight ahead with the off vision perpendicular to the body. The patient was asked to inhale and the horizontal head board was moved down firmly on top of the head and the reading taken to the nearest 0.1cm (Hemsfield *et al,* 1999).

3.9.2 BLOOD COLLECTION AND PROCESSING

About 10ml of blood specimen was collected from a peripheral vein (antecubital venepunture). In this case, the antecubital fossa was cleaned with methylated spirit and allowed to dry. A tourniquet was then applied a few centimeters above the antecubital fossa to detect the veins. Blood was taken with sterile 10ml syringe and 21G needle. This was transferred into a plain bottle and allowed to clot in about 30min. It was centrifuged for 5minutes at 3,000 revolutions per minute. The serum was separated from the cells and transferred to (sample) bottle and then stored frozen at -20 degree celcius until the time for analysis.

3.10 **HORMONAL ASSAYS**

Enzyme Linked Immunosorbent Assay (ELISA) microwell method was used for the quantitative determination of the serum progesterone, prolactin, FSH, LH and oestradiol.

3.10.1 LABORATORY MATERIALS

3.10.1.1Equipment

1. Precision micro pipette 25micrL and 50micrL capacity
2. Microwell reader capable of reading absorbance at 450nm.
3. microwell holder.
4. Device for washing microwells.
5. 37 degree thermostat (incubation chamber).
6. Adhesive film sealant.
7. paper towels or other absorbent materials.
8. Distilled water.
9. Hettich Universal 32 centrifuge (Germany) was used to spin the blood

3.10.1.2 Reagents

The Reagent diagnostic kits for the measurement of serum prolactin, FSH, LH, progesterone and oestradiol were obtained from Syntron Bioresearch.

Inc. 2774 Loker Avenue West. Carlsbad, California United States of America(USA). All the reagents were of analytical grade.

3.10.2 Components of FSH Reagent kit

1.Microwell plate

12 x 8 well strips with break away micro wells coated with anti-monoclonal FSH antibody.

2.Enzyme conjugate

Anti-FSH antibody conjugated with horse-radish peroxidase in a buffer with stabilizer and preservative in a dropper vial.

3.Reference Standard Set

Buffered protein solution with preservative containing FSH in glass vials; 0, 5.0, 10, 25, 50, 100 mIU/ml.

4. Quality Control Set.

Consisted of 2 vial at 1.0ml of control Level I and Control Level II.

5. Dilution Buffer

Buffered protein solution with preservative in a glass vial. To be used for dilution of concentrated samples.

6. **Washing Buffer**

Phosphate Buffer saline in a plastic bottle (30ml) which requires dilution prior to use.

7. **Substrate Reagent A**

0.05M acetate buffer containing 0.02% hydrogen peroxide in a dropper vial.

8. **substrate Reagent B**

3,3',5,5'-Tetramethylbenzidine in stabilizing buffer in a dropper vial.

9. **Stopping Solution**

1N HCl (Hydrochloric acid) in adropper Vial.

3.10.3 COMPONENTS OF LH REAGENT KIT

1. **Microwell plate**

12 x 8 well strips with break away micro wells coated with anti-monoclonal LH antibody.

2. **Enzyme conjugate**

Anti-LH antibody conjugated with horse-radish peroxidase in a buffer with stabilizer and preservative in a dropper vial.

3. **Reference Standard Set**

Buffered protein solution with preservative containing LH in glass vials; 0, 5.0, 10, 25, 50, 100, 200 mIU/ml.

4. **Quality Control Set.**

Consisted of 2 vial at 1.0ml of control Level I and Control Level II.

5. **Washing Buffer**

Phosphate Buffer saline in a plastic bottle (30ml) which requires dilution prior to use.

6. **Substrate Reagent A**

0.05M acetate buffer containing 0.02% hydrogen peroxide in a dropper vial.

7. **Substrate Reagent B**

3,3',5,5'-Tetramethylbenzidine in stabilizing buffer in a dropper vial.

8. **Stopping Solution**

1N HCl(Hydrochloric acid) in a dropper Vial.

3.10.4 Components of Prolactin Reagent kit

1. **Microwell plate**

12 x 8 well strips with break away micro wells coated with anti-monoclonal Prolactin antibody.

2. **Enzyme conjugate**

Anti-Prolactin antibody conjugated with horse-radish peroxidase in a buffer with stabilizer and preservative in a dropper vial.

3. **Reference Standard Set**

Buffered protein solution with preservative containing Prolactin in glass vials; 0, 5.0, 10, 25, 50, 100,200ng/ml.

4. **Quality Control Set.**

Consisted of 2 vial at 1.0ml of control Level I and Control Level II.

5. **Dilution Buffer**

Buffered protein solution with preservative in a glass vial. To be used for dilution of concentrated samples.

6. **Washing Buffer**

Phosphate Buffer saline in a plastic bottle(30ml) which requires dilution prior to use.

7. **Substrate Reagent A**

0.05M acetate buffer containing 0.02% hydrogen peroxide in a dropper vial.

8. **Substrate Reagent B**

3,3',5,5'-Tetramethylbenzidine in stabilising buffer in a dropper vial.

9. **Stopping Solution**

1N HCl(Hydrochloric acid) in a dropper Vial.

3.10.5 Components of Oestradiol Reagent kit

1. **Microwell plate**

12 x 8 well strips with break away micro wells coated with anti-monoclonal Oestradiol antibody.

2. **Enzyme conjugate**

Anti-Oestradiol antibody conjugated with horse-radish peroxidase in a buffer with stabilizer and preservative in a dropper vial.

3. **Reference Standard Set**

Buffered protein solution with preservative containing oestradiol in glass vials; 0, 50, 200, 600, 2000pg/ml.

4. **Quality Control Set.**

Consist of 2 vial at 1.0ml of control Level I and Control Level II.

5. **Dilution Buffer**

Buffered protein solution with preservative in a glass vial. To be used for dilution of concentrated samples.

6. **Washing Buffer**

Phosphate Buffer saline in a plastic bottle(30ml) which requires dilution prior to use.

7. **Substrate Reagent A**

0.05M acetate buffer containing 0.02% hydrogen peroxide in a dropper vial.

8. **Substrate Reagent B**

Buffered chromogen solution with stabiliser, in a dropper vial

9. **Stopping Solution**

1N H_2SO_4(sulphuric acid) in a dropper Vial.

3.10.6 Components of Progesterone Reagent kit

1. **Microwell plate**

12 x 8 well strips with break away micro wells coated with anti-monoclonal Progesterone antibody.

2. **Enzyme conjugate**

Anti-Progesterone antibody conjugated with horse-radish peroxidase in a buffer with stabiliser and preservative in a dropper vial.

3. **Reference Standard Set**

Buffered protein solution with preservative containing Progesterone in glass vials; 0, 0.5, 2.5, 10, 25, 50ng/ml.

4. **.Quality Control Set.**

Consisted of 2 vial at 1.0ml of control Level I and Control Level II.

5. **Dilution Buffer**

Buffered protein solution with preservative in a glass vial. To be used for dilution of concentrated samples.

6. **Washing Buffer**

Phosphate Buffer saline in a plastic bottle(30ml) which requires dilution prior to use.

7. **Substrate Reagent A**

0.05M acetate buffer containing 0.02% hydrogen peroxide in a dropper vial.

8. **Substrate Reagent B**

Buffered chromogen solution with stabiliser, in a dropper vial.

9. **Stopping Solution**

1N HCl(Hydrchloric acid) in a dropper Vial.

3.11 LABORATORYANALYTICAL METHODS:

3.11.1 ESTIMATION OF SERUM FSH

Principles:

The microwell FSH Enzyme Immuno Assay is a solid –phase enzyme immunoassay based on the ‘’sandwich’’ principle. Two separate antibodies directed against distinct antigenic determinants of the FSH molecule are utilised in the assay.

The FSH present in the test sample reacts simultaneously with one antibody immobilised on the micro well surface and with another antbody conjugated to horseradish peroxidase enzyme. So an Ab-Ag-Ab Enyme complex is formed on the microwell surface.

Then the unbound conjugate is removed by washing and the colour development reagents (substrate) are added. Upon exposure to the enzyme, a colour change will take place. The intensity of the colour reflects the amount of bound anti-FSH enzyme conjugate which is proportional to the concentration of FSH in the specimen within the dynamic range of the assay. After stopping the reaction, the resulting colour is measure using a micro well reader at 450nm.

Using a standard curve obtained by ploting the FSH concentrations of reference standards versus the corresponding absorbance, the FSH concentration of the currently run test samples and controls can now be determined.

Assay Procedure

1. A working dilution of washing buffer from the 10x concentrated provided was prepared by mixing 30ml of washing buffer 10x with 270ml of distilled water prior to use.

2. The number of coated microwell needed were determined including an extra well (A1) for substrate blank and a working data sheet with appropriate information was marked.
3. The desired number of microwells from the zip-lock bag were removed and placed in a microwell holder.
4. 50 microL of each reference standards, control and test sample were dispensed into the appropriate well excluding (A1).
5. Two drops of enzyme conjugate were dispensed into each well (excluding A1).
6. The wells were then gently rocked for twenty seconds then sealed by covering with parafilm and incubated at 37^{o}C for 30minutes.
7. The cover slip was then removed and discarded and incubation mixture was thoroughly decanted by flicking into a sink containing disinfectant.
8. The microwells were then wash five times, including A1 with diluted washing Buffer.
9. The wells were dried by firmly tapping the plate on a clean paper towel to remove excess washing solution.

10. One drop of substrate reagents A and B each were then dispense into each well including A1 and the wells were gently rocked for twenty seconds and incubated at room temperature for 10minutes.
11. The reaction was stopped by adding one drop of stopping solution to each well and gently rocking the wells.
12. The absorbance of each well read at 450nm (A_{450}) against the substrate blank(well A1) using microwell reader.

Standard curve and calculation of results

1. Mean absorbance value was calculated for each reference standard, control and patient sample.
2. A standard curve was constructed by plotting the mean absorbance obtained for each reference standard against the concentration in mIU/ml on a graph paper with the absorbance value on the vertical ''Y'' axis and the concentration on the horizontal ''X'' axis.
3. Using the absorbance value for each sample, the corresponding concentration of FSH in mIU/ml was determined from the standard curve.

3.11.2 ESTIMATION OF SERUM LH

Principles:

The microwell LH Enzyme Immuno Assay is a solid –phase enzyme immunoassay based on the ‘’sandwich’’ principle. Two separate antibodies directed against distinct antigenic determinants of the of the LH molecule are utilised in the assay. The LH present in the test sample reacts simultaneously with one antibody immobilised on the micro well surface and with another anti-body conjugated to horseradish peroxidase enzyme. So an Ab-Ag-Ab Enzyme complex is formed on the microwell surface.

Then the unbound conjugate is removed by washing and the colour development reagents (substrate) are added. upon exposure to the enzyme, a colour change will take place. The intensity of the colour reflects the amount of bound anti-LH enzyme conjugate which is proportional to the concentration of LH in the specimen within the dynamic range of the assay.

After stopping the reaction, the resulting colour is measure using a micro well reader at 450nm.

Using a standard curve obtained by ploting the LH concentrations of reference standards versus the corresponding absorbance, the LH concentration of the currently run test samples and controls can now be determined.

Assay Procedure

1 A working dilution of washing buffer from the 10x concentrated provided was prepared by mixing 30ml of washing buffer 10x with 270ml of distilled water prior to use.

2 The number of coated microwell needed were determined including an extra well (A1)for substrate blank and a working data sheet with appropriate information was marked.

3 The desired number of microwells from the zip-lock bag were removed and placed in a microwell holder.

4 50 microL of each reference standards, control and test sample were dispensed into the appropriate well excluding (A1).

5 Two drops of enzyme conjugate were dispensed into each well (excluding A1) .

6 The wells were then gently rocked for twenty seconds then sealed by covering with parafilm and incubated at 37^0 C for 30minutes.

7 The cover slip was then removed and discarded and incubation mixture was thoroughly decanted by flicking into a sink containing disinfectant.

8 The microwells were then washed five times, including A1 with diluted washing Buffer.

9 The wells were dried by firmly tapping the plate on a clean paper towel to remove excess washing solution.

10 One drop of substrate reagents A and B each was then dispensed into each well including A1 and the wells were gently rocked for twenty seconds and incubated at room temperature for 10minutes.

11 The reaction was stopped by adding one drop of stopping solution to each well and gently rocking the wells.

12 The absorbance of each well read at 450nm (A_{450}) against the substrate blank (well A1) using microwell reader.

Standard curve and calculation of results

1. Mean absorbance value was calculated for each reference standard, control and patient sample.
2. A standard curve was constructed by plotting the mean absorbance obtained for each reference standard against the concentration in mIU/ml on a graph paper with the absorbance value on the vertical ‘’Y’’ axis and the concentration on the horizontal ‘’X’’ axis.

3. Using the absorbance value for each sample, the corresponding concentration of FSH in mIU/ml was determined from the standard curve.

3.11.3 ESTIMATION OF SERUM PROLACTIN

Principles:

The microwell Prolactin Enzyme Immuno Assay is a solid –phase enyme immunoassay based on the ‘’sandwich’’ principle. Two separate antibodies directed against distinct antigenic determinants of the of the Prolactin molecule are utilised in the assay. The Prolactin present in the test sample reacts simultaneously with one antibody immobilised on the micro well surface and with another antbody conjugated to horseradish peroxidase enzyme. So an Ab-Ag-Ab Enzyme complex is formed on the microwell surface.

Then the unbound conjugate is removed by washing and the colour development reagents (substrate) are added. Upon exposure to the enzyme, a colour change will take place. The intensity of the colour reflects the amount of bound anti-Prolactin enzyme conjugate which is proportional to the concentration of Prolactin in the specimen within the dynamic range of the assay. After stopping the reaction, the resulting colour is measured using a micro well reader at 450nm.

Using a standard curve obtained by ploting the Prolactin concentrations of reference standards versus the corresponding absorbance, the Prolactin concentration of the currently run test samples and controls can now be determined.

Assay Procedure

1. A working dilution of washing buffer from the 10x concentrated provided was prepared by mixing 30ml of washing buffer 10x with 270ml of distilled water prior to use.

2. The number coated microwell were determined including an extra well (A1) for substrate blank and a working data sheet with appropriate information was marked.

3. The desired number of microwells from the zip-lock bag were removed and placed in a microwell holder.

4. 50 microL of each reference standards, control and test sample were dispensed into the appropriate well excluding (A1).

5. Two drops of enzyme conjugate were dispensed into each well (excluding A1) .

6 The wells were then gently rocked for twenty seconds then sealed by covering with parafilm and incubated at 37^0 C for 30minutes.

7 The cover slip was then removed and discarded and incubation mixture was thoroughly decanted by flicking into a sink containing disinfectant.

8 The microwells were then washed five times, including A1 with diluted washing Buffer.

9 The wells were dried by firmly tapping the plate on a clean paper towel to remove excess washing solution.

10 One drop of substrate reagents A and B each were then dispense into each well including A1 and the wells were gently rocked for twenty seconds and incubated at room temperature for 10minutes.

11 The reaction was stopped by adding one drop of stopping solution to each well and gently rocking the wells.

12. Absorbance of each well was read at 450nm(A_{450})against the Substate blank(well A1) using microwell reader.

Standard curve and calculation of results

1. Mean absorbance value was calculated for each reference standard, control and patient sample.
2. A standard curve was constructed by plotting the mean absorbance obtained for each reference standard against the concentration in ng/ml on a graph paper with the absorbance value on the vertical ''Y'' axis and the concentration on the horizontal ''X'' axis.
3. Using the absorbance value for each sample, the corresponding concentration of prolactin in ng/ml was determined from the standard curve.

3.11.4 ESTIMATION OF SERUM OESTRADIOL(E_2)

Principles:

The microwell Oestradiol (E_2) Enzyme Immuno Assay is a solid –phase enzyme immunoassay based on the competitive binding principle. The Oestradiol present in the test sample will compete with enzyme –labelled oestradiol for binding rabit anti- E_2 antibody linked with goat anti-rabit, immobilised on the microwell surface.The amount of conjugate that binds to the anti-E_2 antibody is inversely proportional to the concentration of E_2 in the patient sample.

The unbound sample and conjugate are then removed by washing and the colour development reagents (substrate) are added. Upon exposure to the bound enzyme, a colour change will take place. The intensity of the colour reflects the amount of bound enzyme –**E_2** conjugate and is inversely proportional to the concentration of oestradiol E_2 in the specimen within the dynamic range of the assay. After stopping the reaction, the resulting colour is measure using a micro well reader at 450nm. Using a standard curve obtained by ploting the oestradiol concentrations of reference standards versus the corresponding absorbance, the oestradiol concentration of the currently run test samples and controls can now be determined.

Assay Procedure

1.A working dilution of washing buffer from the 10x concentrated provided was prepared by mixing 30ml of washing buffer 10x with 270ml of distilled water prior to use.

2. The number coated microwell were determined including an extra well (A1) for substrate blank and a working data sheet with appropriate information was marked.

3. The desired number of microwells from the zip-lock bag were remove and placed in a microwell holder.
4. 50 microL of each reference standards, control and test sample were dispensed into the appropriate well.
5. One drop of enzyme conjugate were dispensed into each well.
6. The wells were then gently rocked for twenty seconds then sealed by covering with parafilm and incubated at 37^0 C for 60minutes.
7. The cover slip was then removed and discarded and incubation mixture was thoroughly decanted by flicking into a sink containing disinfectant.
8. The microwells were then washed three times, with the diluted washing Buffer.
9. The wells were dried by firmly tapping the plate on a clean paper towel to remove excess washing solution.
10. One drop of substrate reagents A and B each were then dispense into each well and the wells were gently rocked for twenty seconds and incubated at room temperature for 15minutes in the dark.

11.The reaction was stopped by adding one drop of stopping solution to each well and gently rocking the wells.

12.The absorbance of each well read at 450nm(A_{450}) using microwell reader.

Standard curve and calculation of results

1. Mean absorbance value was calculated for each reference standard, control and patient sample.
2. The average value A_{450} value for each standard , control and test sample were divided by the average A_{450} of standard 0 pg/ml and multiply by 100 to obtain % A/Ao for each sample.
3. A standard curve was constructed by plotting the % A/Ao value of each standard against the corresponding concentrations of the standard on a graph paper.
4. Using the absorbance or the % A/Ao value for each control and test sample, the corresponding concentration of estradiol in pg/ml was determined from the standard curve.

3.11.5 ESTIMATION OF SERUM PROGESTERONE

Principles:

The microwell progesterone Enzyme Immuno Assay is a solid –phase enzyme immunoassay based on the competitive binding principle. The progesterone present in the test sample will compete with enzyme –labelled progesterone conjugate for binding with anti- progesterone antibody immobilised on microwell surface. The amount of conjugate that binds to the microwell surface will decrease in proportion to the concentration of progesterone in the patient's sample.

The unbound sample and conjugate are then removed by washing and the colour development reagents (substrate) are added. Upon exposure to the bound enzyme, a colour change will take place. The intensity of the colour reflects the amount of bound enzyme –progesterone conjugate and is inversely proportional to the concentration of progesterone in the patient sample within the dynamic range of the assay. After stopping the reaction, the resulting colour is measured using a micro well reader at 450nm. Using a standard curve obtained by ploting the Progesterone concentrations of reference standards versus the corresponding absorbance, the progesterone concentration of the currently run test samples and controls can now be determined.

Assay Procedure

1. A working dilution of washing buffer from the 10x concentrated provided was prepared by mixing 30ml of washing buffer 10x with 270ml of distilled water prior to use.
2. The number of coated microwell needed were determined including extra well (A1) for substrate blank and a working data sheet with appropriate information was marked.
3. The desired number of microwells from the zip-lock bag were removed and placed in a microwell holder.
4. 50 microL of each reference standards, control and test sample were dispensed into the appropriate well.
5. One drop of enzyme conjugate was dispensed into each well.
6. The wells were then gently rocked for twenty seconds then sealed by covering with parafilm and incubated at 37^{o}C for 60minute.
7. The cover slip was then removed and discarded and incubation mixture was thoroughly decanted by flicking into a sink containing disinfectant.

8. The microwells were then washed five times, with the diluted washing Buffer.
9. The wells were dried by firmly tapping the plate on a clean paper towel to remove excess washing solution.
10. One drop of substrate reagents A and B each were then dispensed into each well and the wells were gently rocked for twenty seconds and incubated at room temperature for 15minutes in the dark.
11. The reaction was stopped by adding one drop of stopping solution to each well and gently rocking the wells.
12. The absorbance of each well was read at 450nm(A_{450}) using microwell reader.

Standard curve and calculation of results

1. Mean absorbance value was calculated for each reference standard, control and patient sample.
2. The average value A_{450} value for each standard , control and test sample were divided by the average A_{450} of standard 0 ng/ml and multiply by 100 to obtain % A/Ao for each sample.

3. A standard curve was constructed by plotting the % A/A_o value of each standard against the corresponding concentrations of the standard graph paper.

4. Using the absorbance or the % A/Ao value for each control and test sample, the corresponding concentration of progesterone in ng/ml was determined from the standard curve.

3.12 PRECISION STUDIES;

Three levels of control samples(low, medium and high) were selected and assay in duplicates. The replicate determination of the two different control sera were done in one assay (within – run precision) and in five different assays (between – run precision). The precision was evaluated by calculating the mean, standard deviation and coefficient of variation for each level of control sample.

3.13 STATISTICAL ANALYSIS

The mean of all analytes measured including their standard deviation (mean + SD) were determined. This was done for both patients and control subjects.

Differences in serum hormone levels between the groups of fertile and infertile men were tested by student t test. A p- value of equal to or less than 0.05($p \leq 0.05$) was considered as statistically significant. The statistical package for the social science (SPSS) for window version 15.0 was used for all calculations and statistical analysis.

3.13 PROBLEMS ENCOUNTERED DURING THE EXECUTION OF THE PROJECT.

The subjects were told specifically to come in a fasting state, as well as not having exercised for 12 hours prior to their consultations. The biggest problem encountered was subjects coming in for appointments and not being in a fasting state. These subjects were then asked to make other appointments, so that all the measurements could be taken while they were in a fasting state. None of these patients turned up for their appointments. This affected the total number of subjects recruited especially the control groups and delayed the study.

CHAPTER FOUR

RESULTS

4.1 Analysis of Study Population

A total of one hundred and eighty (180) subjects were recruited for the study aged 18 to 44 years . These were made up of one hundred and twenty 120 infertile women and sixty 60 fertile women as controls.

They were divided into three age group as shown in table 4.1 below. Majority of the infertile women 75(62.5%) and the controls 30(50%) in this present study were in the age group 25-34 .

TABLE 4.1: Shows the age group distribution of the infertile women and controls.

	frequency		Percentage (%)	
Age group in years	Infertile women	Controls	Infertile women	controls
15-24	11	10	9.2	17.0
25-34	75	30	62.5	50.0
35-44	34	20	28.3	33.0
Total	120	60	100	100

The mean duration of infertility was 5years. 38 (31.7%) had primary infertility and 82 (68.3%) had secondary infertility.The major educational status of the infertile women was tertiary education which constitutes 74(61.7%) of the infertile women and their major occupation was civil servant which constitutes 51(42.5%) , while in contrast the major educational status of the fertile control women was secondary education which constitutes 22 (36.7%) and their major occupation was house wife which constitutes 31(51.7%) of the fertile control women.The infertile women were made up of 85 (70.8%) in follicular phase , and 29 (24.2%) in luteal phase of their menstrual cycles and 6 (5%) were amenorrheic (mainly with secondary amenorrhoea). The fertile women (control) were made up of 35 (58.3%) in follicular phase, and 25 (47.7%) in luteal phae of their menstrual cycles. Majority of the infertile women (71.7%) were either overweight or obese while majority of the controls (56.6%) were within normal weight based on their body mass index (BMI). (Table 4.2)

Table 4.2: The frequency distributions of some clinical parameters of the infertile women and controls.

	Frequency (n)		Percentage (%)	
Characteristics	**Infertile women**	**controls**	**Infertile women**	**Controls**
a)**Types of infertility**				
primary	38	-	31.7	-
secondary	82	-	68.3	-
b)**Educational status**				
Tertiary	74	15	61.7	25.0
Secondary	28	23	23.3	36.0
Primary	11	7	9.2	11.7
Non formal	7	16	5.8	26.7
c) Occupation				
Civil servants	51	15	42.5	25.7
Self employed	19	10	15.8	16.7
Students	12	4	10.0	6.7
Housewives	38	31	31.7	51.7
d)Menstrual history				
Follicular phase	85	35	70.8	58.3
Luteal phase	29	25	24.2	41.7
Amenorrheic	6	-	5	-
e)BMI(kg/m^2)				
<18.5 underweight	-	-	-	-
18.5-24.9 Normal	34	34	28.3	56.7
25.0-29.9 overweight	44	13	36.7	21.7
30.0-34.9 Obese I	29	8	24.2	13.3
35.0-39.9 Obese II	10	4	8.3	6.7
≥40.0 ObeseIII	3	1	2.5	1.7

4.2: The clinical parameters in infertile women and controls.

The clinical parameters in the infertile women and the matched controls are presented in table 4.3. The mean ages, age at menarche, mean duration of menstrual flow and the blood pressure in the infertile women were not statistically different from those in controls ($p>0.05$). The mean body mass index (BMI) and the mean duration of menstrual cycles were significantly higher ($p<0.05$) in infertile women than in controls. The mean parity was significantly lower ($p<0.05$) in infertile women than in controls.

.

TABLE 4.3: Clinical Parameters of control subjects and infertile women.

Characteristics	Infertile women Mean (Ranges)	Controls Mean (Ranges)	P values
Age(yr)	31(19 – 45)	30.5(18 – 42)	>0.05
Age at menarche	14.2(10 – 25)	14.4(12 – 18)	>0.05
Parity	0.8(0 – 5)	5(1 – 13)	<0.05
Dur. Menst.Cycles(days)	64.5(17.5 – 278)	26.7(21 – 30)	<0.05
Dur.menst. fl (days)	4.3(2 – 8)	4.6(2.5 – 8.5)	>0.05
Body mass index(kg/m^2)	28.4(18.8 – 56)	25.1 (15.4 – 42)	<0.05

4.3: **Perfomance Characteristics and Calibrations of ELISA Techniques for Hormonal Assays.**

4.3.1 Calibration of the method for serum LH

Table 4.4 shows absorption measurements in duplicates on a multiskan microplate reader for series of LH standards. A typical plot of the absorbance against the concentration of the LH standards (mIU/ml) is shown in fig 4.1. The linear curve shows a positively significant correlation ($r=0.970$, $t=2.034$, $P<0.05$) .

4.3.2 Precision profiles for LH

The results of within –runs and between runs precision studies are presented in Table 4.5:

The within –runs coefficient of variation for low, medium and high concentration were 7.9%, 8.0% and 5.9% respectively while the corresponding values for the between - runs were 9.7%, 9.8% and 6.2% respectively.

Table 4.4: calibration of the method for the measurement of concentration of LH on multiskan microplate reader at 450nm.

Standard	1	2	3	4	5	6	7
Conc.LH(mIU/ml)	0	5	10	25	50	100	200
1st Absorbance (nm)	0.01	0.16	0.25	0.55	0.99	1.62	2.20
2nd Absorbance (nm)	0.01	0.12	0.24	0.59	1.01	1.602	2.24
Average (nm)	0.01	0.14	0.24	0.57	1.00	1.59	2.22

Table 4.5: Precision profile for measuring serum LH on multiskan microplate reader.

$\overline{LH}$	n	Within- Runs Precision X mIU/ml	SD mIU/ml	CV %	n	Between- Runs Precision X mIU/ml	SD mIU/ml	CV %
$\overline{LOW}$	10	10	7.9	7.9	10	9.7	0.9	9.7
MED	10	32.2	2.6	8.0	10	35.0	3.4	9.8
HIGH	10	160.9	9.3	5.9	10	156.5	9.7	6.2

n= number of estimations; X=Mean; SD=Standard deviation
CV= Coefficient of variation

r=0.0970, t=2.034,
p<0.05

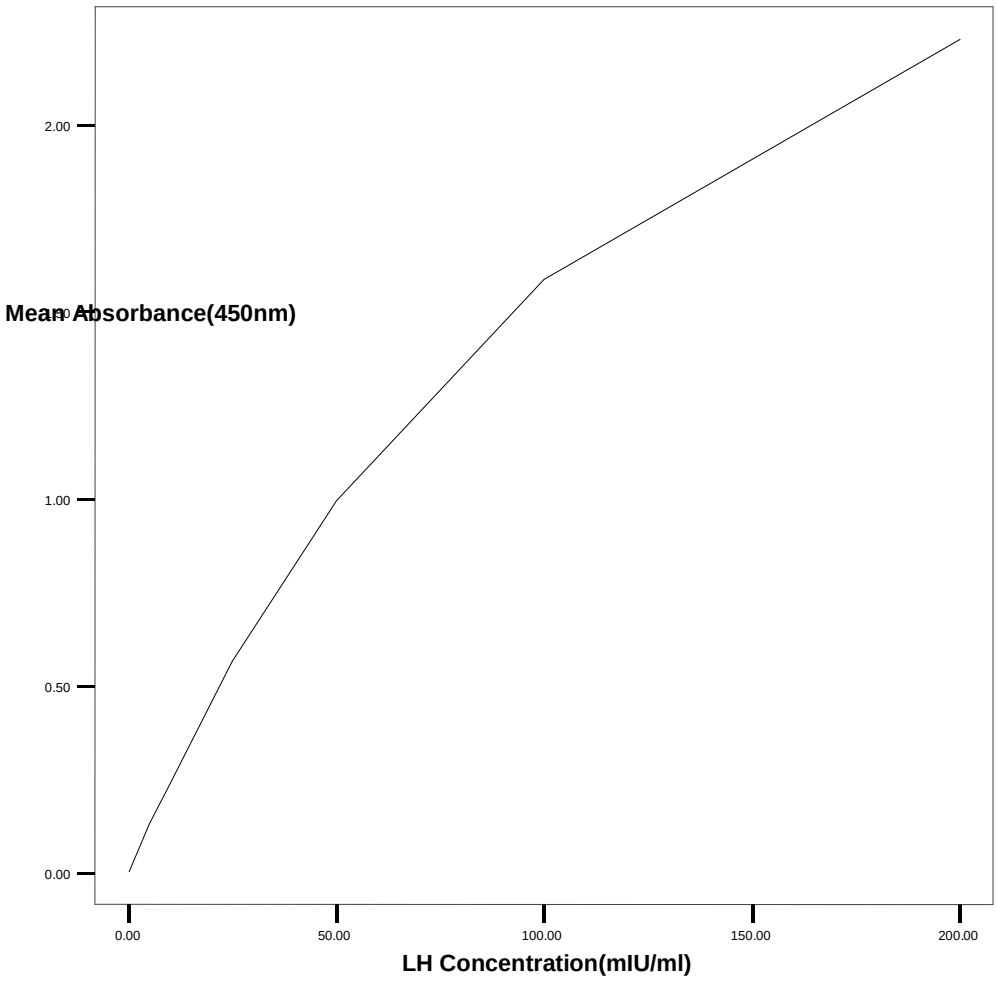


Fig. 4.1: Standard calibration Curve for serum LH on multiskan Microplate Reader.

The corresponding concentrations of LH in mIU/ml was determined from the standard curve for each sample

4.3.3: Calibration of the method for serum FSH

Table 4.6 shows absorption measurements in duplicates on a multiskan microplate reader for series of FSH standards. A typical plot of the absorbance against the concentration of the FSH standards (mIU/ml) is shown in fig 4.2. The linear curve shows a positively significant correlation ($r = 0.999$, $t = 2.039$, $P<0.05$)

4.3.4: Precision profiles for FSH

The results of within –runs and between runs precision studies are presented in Table 4.7.

The within –runs coefficient of variation for low, medium and high concentration were 7.2%, 7.3% and 8.4% respectively while the corresponding values for the between - runs were 8.6%, 5.5% and 9.0% respectively.

Table 4.6: calibration of the method for the measurement of concentration of F SH on multiskan microplate reader at 450nm.

Standard	1	2	3	4	5	6
Conc.(mIU/ml)	0	5	10	25	50	100
1st Absorbance (nm)	0.02	0.12	0.26	0.63	1.12	2.24
2nd Absorbance (nm)	0.06	0.12	0.22	0.55	1.22	2.16
Average (nm)	0.04	0.12	0.24	0.59	1.17	2.20

Table 4.7: Precision profile for measuring serum FSH on multiskan microplate reader.

FSH.	n	Within- Runs Precision X mIU/ml	SD mIU/ml	CV %	n	Between- Runs Precision X mIU/ml	SD mIU/ml	CV %
LOW	10	7.1	0.5	7.2	10	6.9	0.6	8.6
MED	10	16.8	1.2	7.3	10	16.9	0.9	5.5
HIGH	10	48.1	4.0	8.4	10	49.2	4.4	9.0

n= number of estimations; X= mean; SD= Standard deviation;
CV= coefficient of variation

r =0.999
t =2.039
p < 0.05

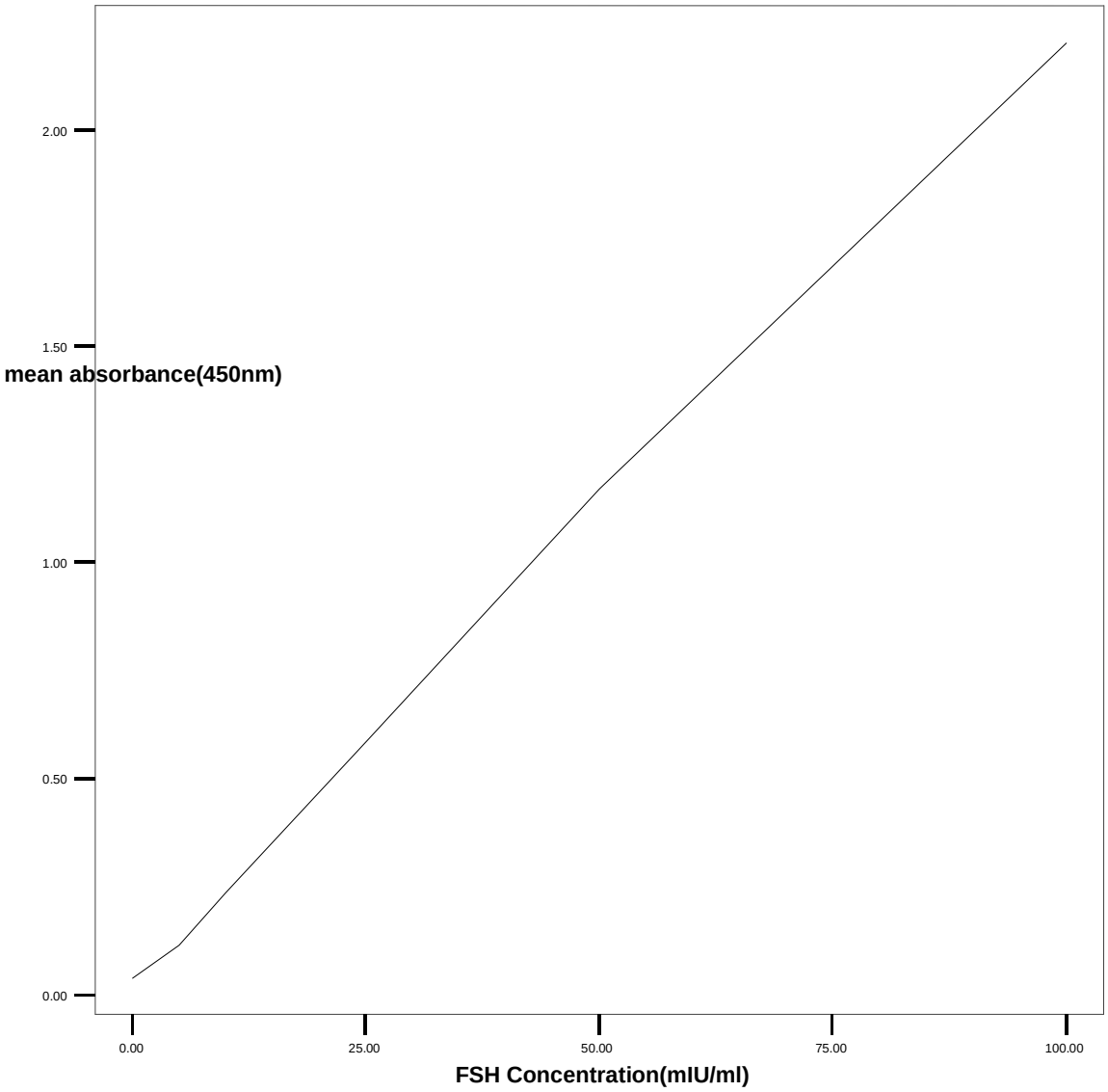


Fig 4.2: Standard calibration Curve for serum FSH on multiskan Microplate Reader. The corresponding concentrations of FSH in mIU/ml was determined from the standard curve for each sample.

4.3.5: Calibration of the method for serum Prolactin

Table 4.8 shows absorption measurements in duplicates on a multiskan microplate reader for series of Prolactin standards . A typical plot of the absorbance against the concentration of the FSH standards (ng/ml) is shown in fig 4.3. The linear curve shows a positively significant correlation ($r=0.973$, $t=2.034$, $p<0.05$).

4.3.6: Precision profiles for serum Prolactin

The results of within –runs and between runs precision studies are presented in Table 4.9.

.

The within –runs coefficient of variation for low, medium and high concentration were 7.5%, 5.6% and 3.1% respectively while the corresponding values for the between-runs were 9.3%, 7.4% and 6.9% respectively.

Table 4.8: calibration of the method for the measurement of concentration of prolactin on multiskan microplate reader at 450nm.

Standard	1	2	3	4	5	6	7
Conc.(ng/ml)	0	5	10	25	50	100	200
1st Absorbance (nm)	0.02	0.15	0.03	0.60	1.03	1.62	2.24
2nd Absorbance (nm)	0.04	0.19	0.03	0.70	0.98	1.62	2.32
Average (nm)	0.03	0.17	0.30	0.60	1.01	1.62	2.28

Table 4.9: Precision profile for measuring serum prolactin on multiskan microplate reader.

PRL	n	Within- Runs Precision			n	Between- Runs Precision		
		X mIU/ml	SD mIU/ml	CV %		X mIU/ml	SD mIU/ml	CV %
LOW	10	7.5	0.6	7.5	10	7.5	0.7	9.3
MED	10	17.0	0.9	5.6	10	16.2	1.2	7.4
HIGH	10	43.3	1.3	3.1	10	46.5	3.2	6.9

n = number of estimations; X= Mean; SD=Standard deviation; CV=Coefficient of variation.

r =0.973
t=2.034
P < 0.05

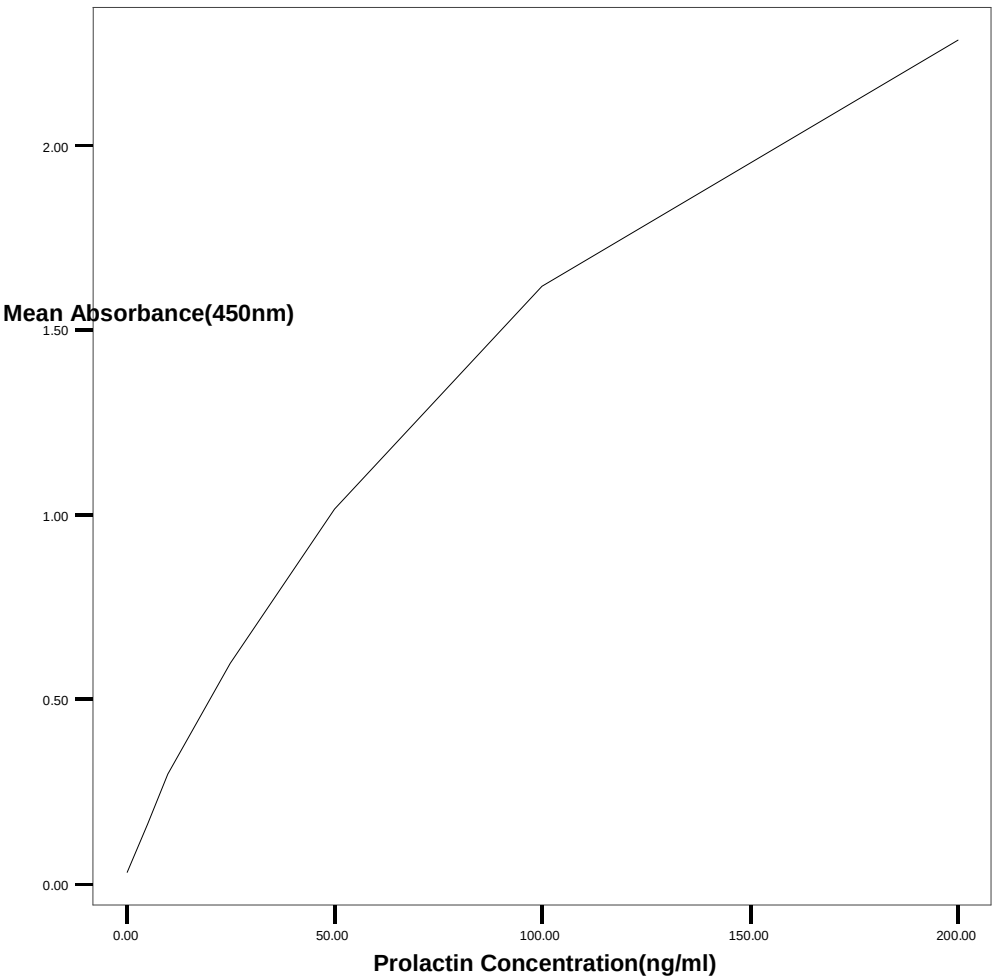


Fig.4.3: Standard calibration Curve for serum Prolactin on multiskan Microplate Reader.

The corresponding concentrations of Prolactin in ng/ml determined from the standard curve for each sample.

4.3.7: Calibration of the method for serum Progesterone

Table 4.10 shows absorption measurements in duplicates on a multiskan microplate reader for series of progesterone standards. A typical plot of the absorbance against the concentration of the progesterone standards (ng/ml) is shown in fig 4.4 The linear curve shows a negatively significant correlation ($r = -0.826$, $t = 1.823$, $p < 0.05$).

4.3.8: Precision profiles for Progesterone

The results of within –runs and between runs precision studies are presented in Table 4.11.

The within –runs coefficient of variation for low, medium and high concentration were 7.5%, 5.7% and 3.1% respectively while the corresponding values for the between - runs were 9.5%, 7.3% and 6.9% respectively.

Table 4.10: calibration of the method for the measurement of concentration of progesterone on multiskan microplate reader at 450nm.

Standard	0	1	2	3	4	5
Conc.(ng/ml)	0.0	0.5	2.5	10	25	50
1st Absorbance (nm)	2.04	1.69	1.25	0.69	0.43	0.30
2nd Absorbance (nm)	2.08	1.65	1.25	0.75	0.43	0.28
Average (nm)	2.06	1.67	1.25	0.72	0.43	0.29

Table 4.11: Precision profile for measuring serum progesterone on multiskan microplate reader.

PROG	n	Within- Runs Precision			n	Between- Runs Precision		
		X mIU/ml	SD mIU/ml	CV %		X mIU/ml	SD mIU/ml	CV %
LOW	10	7.5	0.6	7.5	10	7.5	0.7	9.5
MED	10	17	0.97	5.7	10	16.2	1.2	7.3
HIGH	10	43.3	1.3	3.1	10	46.5	3.2	6.9

n= number of estimations; X= Mean; SD = Standard deviation;
CV = coefficient of variation

r = - 0.826,
t = 1.823,
p < 0.05

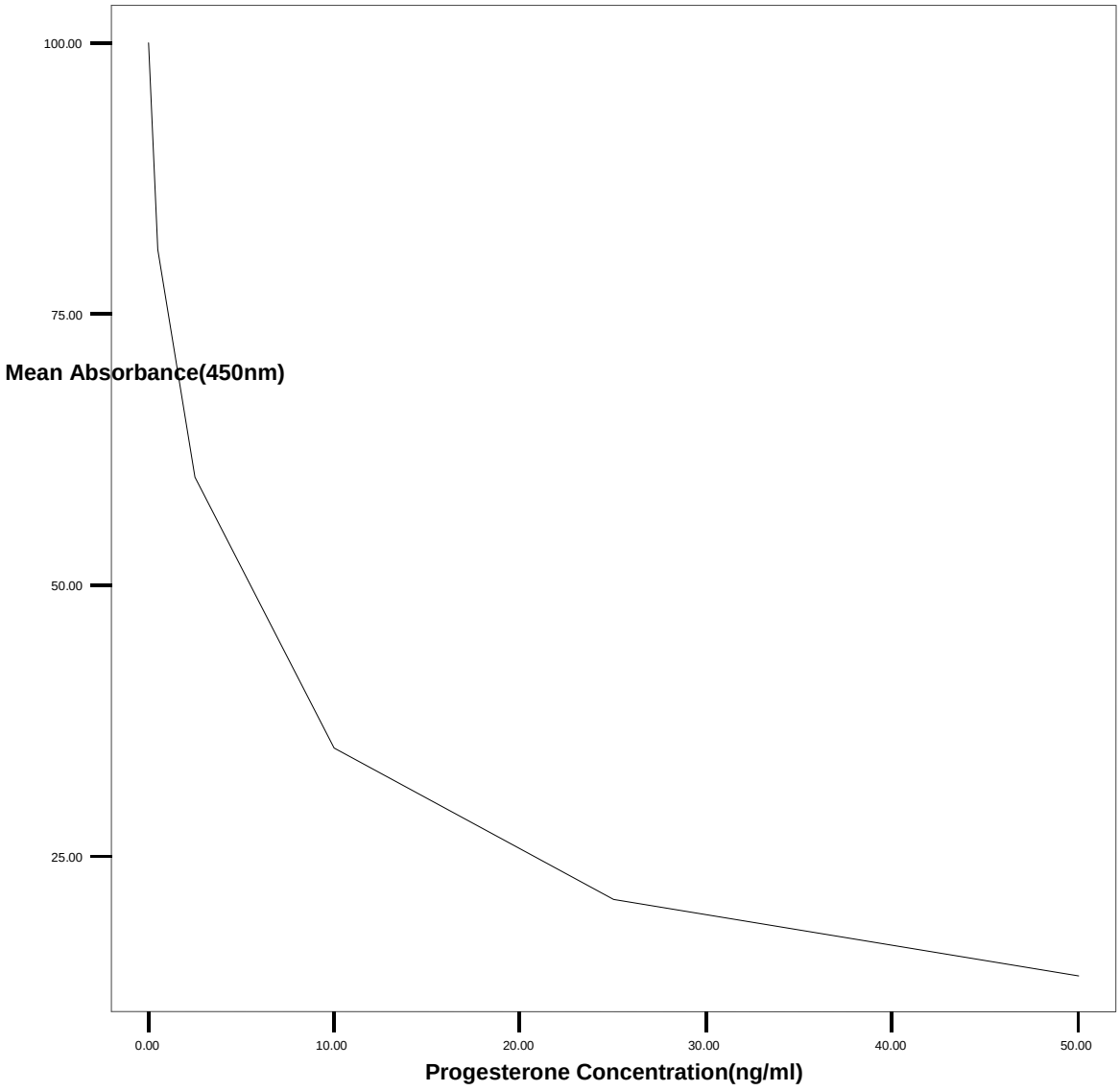


Fig 4.4: Standard calibration curve for Serum Progesterone on multiskan Microplate Reader.
The corresponding concentrations of Progesterone in ng/ml was determined from the standard curve for each sample.

4.3.9: Calibration of the method for serum Oestradiol.

Table 4.12 shows absorption measurements in duplicate on a multiskan microplate reader for series of Oestradiol standards (pg/ml). A typical plot of the absorbance against the concentration of the Oestradiol standards (pg/ml) is shown in fig 4.5. The linear curve shows a negatively significant correlation ($r = -0.881$, $t = 1.529$, $p < 0.05$).

4.3.10: Precision profiles for oestradiol.

The results of within –runs and between runs precision studies are presented in Table 4.14:

The within –run coefficient of variation for low, medium and high concentration were 5.1%, 2.9% and 2.1% respectively while the corresponding values for the between-runs were 10.1%, 5.3% and 2.7% respectively.

Table 4.12: calibration of the method for the measurement of concentration of oestradiol on multiskan microplate reader at 450nm.

Standard	1	2	3	4	5
Conc.(pg/ml)	0	50	200	600	2000
1st Absorbance (nm)	2.14	1.80	1.48	1.16	0.78
2nd Absorbance (nm)	2.10	1.76	1.48	1.12	0.74
Average (nm)	2.12	1.78	1.48	1.14	0.76

Table 4.13: Precision profile for measuring serum oestradiol on multiskan microplate reader.

E_2	n	Within- Runs Precision			n	Between- Runs Precision		
		X mIU/ml	SD mIU/ml	CV %		X mIU/ml	SD mIU/ml	CV %
LOW	10	148.3	7.6	5.1	10	119.1	12.0	10.1
MED	10	524.9	5.1	2.9	10	438.1	23.3	5.3
HIGH	10	1861.4	40.0	2.1	10	1742.0	47.6	2.7

n = Number of estimations; X= Mean; SD= Standard deviation;
CV= Coefficient of variation; E_2 = Oestradiol

r = - 0.881
t = 1.529
p < 0.05

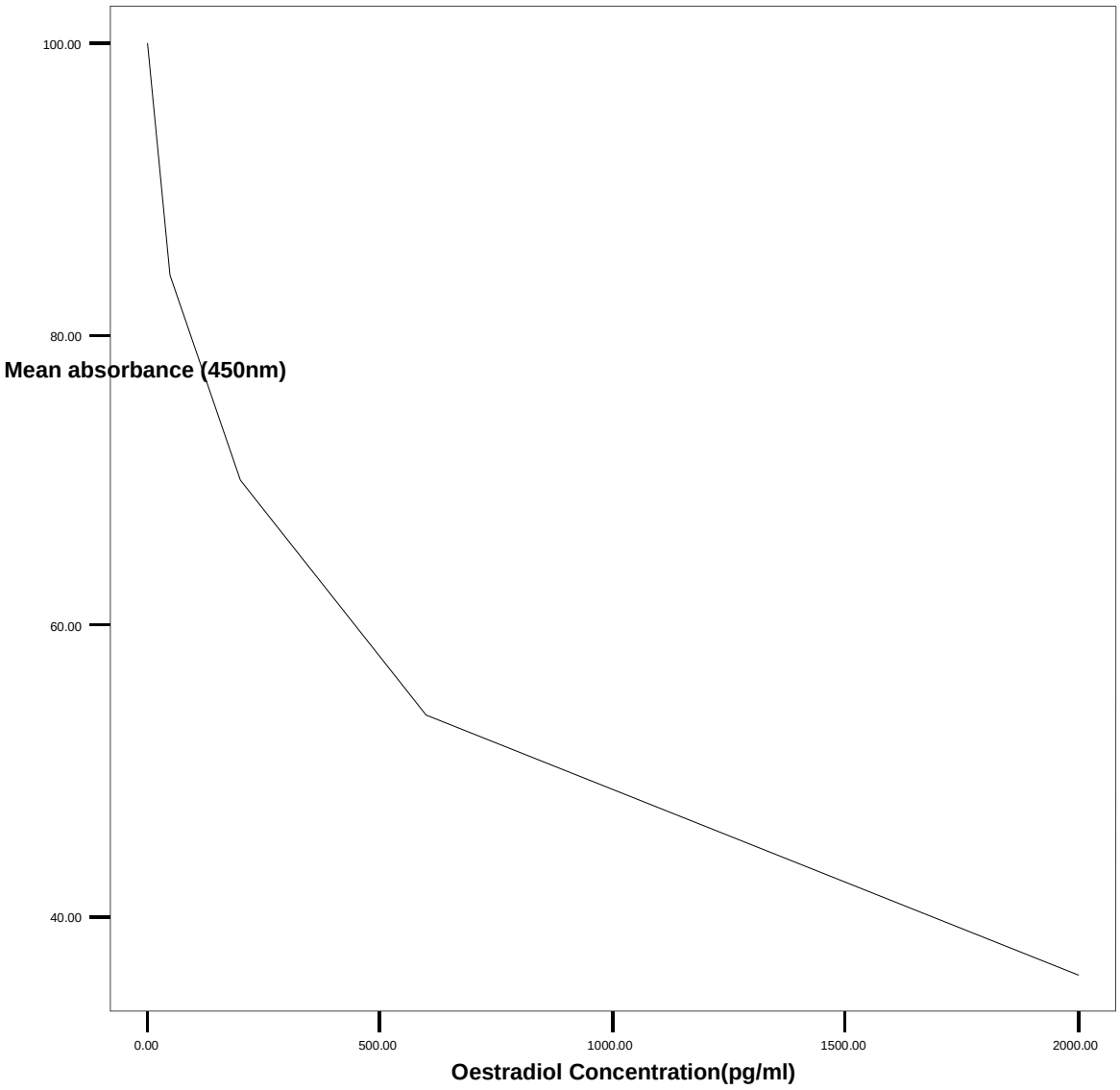


Fig.4.5: Standard calibration Curve for serum Oestradiol on multiskan Microplate Reader.

The corresponding concentrations of Oestradiol in pg/ml was determined from the standard curve for each sample.

4.4: Serum Hormonal Profile in Infertile women and controls.

The mean values for LH, FSH, Prolactin,progesterone, oestradiol, and LH/FSH ratio in infertile women and control are presented in table 4.14 Serum LH, Prolactin and LH/FSH ratio were statistically higher ($P< 0.05$) in infertile women than controls, while the serum progesterone and oestradiol in infertile women were significantly lower ($P<0.05$) than in control. There is no significant statistical difference between the FSH of the infertile women and controls ($p>0.05$).

4.5: Serum hormonal Profile in Infertile women with primary infertility compared to infertile women with secondary infertility.

The mean values for LH, FSH, Prolactin,progesterone, oestradiol, and LH/FSH in infertile women with primary infertility and secondary infertility are presented in table 4.15:

The serum LH, FSH, prolactin, progesterone, oestradiol and LH/FSH were not significantly different ($P> 0.05$).

TABLE 4.14 Biochemical Parameters of control subjects and infertile women.

Reproductive Hormones	Infertile women Mean (Range)	Controls mean (Range)	P-values
LH (mIU/ml)	34.7 (2-174)	12.3(3-28)	<0.05
FSH(mIU/ml)	14.1(0.5-67)	13(3.8-39)	>0.05
Prolactin (ng/ml)	31(2.5-109)	8.7(0.15-22)	<0.05
Progesterone (ng/ml)	5.4(0.2-75)	17.8(3-75)	<0.05
Oestradiol(pg/ml)	188.2(0.4-820)	366(22-1040)	<0.05
LH/FSH ratio	4(0.2-56)	2.4(0.5-12)	<0.05

TABLE 4.15 : SERUM HORMONAL PROFILE (MEAN±2SD) IN INFERTILE WOMEN WITH PRIMARY INFERTILITY COMPARE TO INFERTILE WOMEN WITH SECONDARY INFERTILTY.

Subjects	n	LH mIU/ml	FSH mIU/ml	PRL ng/ml	Prog ng/ml	E_2 pg/ml	LH/FSH ratio
prim.	38	30.7± 29.5	13.8± 12.2	31.4± 22.4	6.5± 13.2	213± 176	3±2.2
sec.	82	36.6± 26.4	14.3± 11.0	30.7 ±19.3	4.8 ±10.1	176.6± 147	4.4±6.8
P- value		> 0.05	>0.05	>0.05	>0.05	<0.05	>0.05

LH= Leutinizing hormone; FSH= Follicle Stimulating hormone; PRL= Prolactin; E_2=Estradiol; prim= primary infertility; sec= secondary infertility; prog= Progesterone; n=number of subjects.

4.6: Pattern of hormonal profile in infertile women

Table 4.16 shows the pattern of the hormonal profile (LH, FSH, LH/FSH ratio, prolactin, progesterone and oestradiol) in infertile women. Subjects with elevated LH, prolactin, LH/FSH ratio, low progesterone and oestradiol are the commonest features in most infertile women. Elevated prolactin level was found to coexist with some other hormonal disorders.

Table 4.17 shows the hormonal disorders with possible diagnosis in the infertile women.

Polycystic ovarian Syndrome (PCOS) with hyperprolactinaemia 33.3% have the highest incidence among the infertile women. There were multiple endocrine disorders and hyperprolactinaemia was found to coexist with some other hormonal disorders such as polycystic ovarian syndrome , luteal phase deficiency, Hypergonadotropic hypergonadism and hypergonadotropic hypogonadism. 28.3% of the infertile women were found to have no hormonal abnormalities.

Table 4.18 shows the percentage of the total major hormonal abnormalities in infertile women. Hyperprolactinaemia 50% was found to have the highest incidence while hypergonadotropic hypergonadism 1.7% and hypogonadotropic hypogonadism 1.7% were found to have the lowest incidence.

TABLE 4.16: Pattern of Hormonal Profile in Infertile Women

LH	FSH	LH/FSH	Prl	Progest	E2	n	%	Findings
high	N/low	high	high	low	low	40	33.3	High LH, LH/FSH, Prl, low progest;E_2
normal	normal	normal	high	low	normal	16	13.3	High Prl, low progest
high	N/low	high	normal	low	low	16	13.3	High LH, LH/FSH, low progest,E_2
normal	normal	normal	normal	low	normal	3	2.5	Low progest.
normal	normal	normal	normal	normal	normal	34	28.3	Normal hormonal levels
high	high	normal	normal	low	normal	5	4.2	High LH,FSH,low progest
high	high	normal	high	low	normal	2	1.7	High LH,FSH, Prl, low progest
high	high	normal	high	high	normal	2	1.7	High LH, FSH,Prl, progest.
low	low	normal	normal	low	normal	2	1.7	Low LH,FSH,Progest.

Prl= Prolactin; Progest= Progesterone; LH= Lutenizing Hormone; FSH=Follicle Stimulating Hormone; E_2= Oestradiol; n= Number of infertile women; %= percentage.

TABLE 4.17: Specific Hormnal Disorders in Infertile Women

Findings	% of Infertile Women	Possible Diagnosis
High LH, LH/FSH, Prl, low progest;E_2	33.3	Polycystic ovarian syndrome and hyperprolactinaemia
High Prl, low progest	13.3	Hyperprolactinaemia and luteal phase defficiency.
High LH, LH/FSH, low progest,E_2	13.3	Polycystic ovarian syndrome
Low progest.	2.5	Luteal phase deficiency
Normal hormonal levels	28.3	No hormonal disorder
High LH,FSH,low progest	4.2	Hpergonadotropic hypogonadism
High LH,FSH, Prl, low progest	1.7	Hypergonadotropic hypogonadism and hyperprolactinaemia
High LH, FSH,Prl, progest.	1.7	Hypergonadotropic hypergonadism and hyperprolactinaemia
Low LH,FSH,Progest.	1.7	Hypogonadotropic hypogonadism
Total	100	

Prl= Prolactin; Progest= Progesterone; LH= Lutenizing Hormone; FSH=Follicle Stimulating Hormone; E_2= Oestradiol; %= percentage.

Table 4.18: Total percentage of major hormonal disorders in infertile women

Hormonal disorders	n	percentage(%)
Hyperprolactinaemia	60	50
Polycystic ovarian syndrome	56	46.6
Luteal phase deficiency	19	15.8
Hypergonadotropic hypogonadism	7	5.9
Hypogonadotropic hypogonadism	2	1.7
Hypergonadotopic hypergonadism	2	1.7

4.7: Relationship between BMI and Biochemical parameters in infertile women and controls

There was no significant correlation between `BMI and serum LH in the infertile women (r = 0.102, p=0.269) while there was a significant positive correlation between BMI and serum LH in controls(r=0.263, p=0.043) as shown in fig.4.6. There were no significant correlations between BMI and serum FSH in both the infertile women(r= -0.003, p= 0.972) and controls (r=0.088, p=0.503). Similarly there were no significant correlations between BMI and serum prolactin in both the infertile women (r=0.003, p=0.970) and the controls(r=0.007, p=0.607).There were also no significant correlations between BMI and serum progesterone in both infertile women (r= -0.127, p=0.168) and the controls (r=0.189, p= 0.148). No significant correlations were found between BMI and serum oestradiol in both infertile women (r= 0.015, p=0.874) and the controls (r= -0.107, p=0.414).

No significant correlations were found between BMI and serum LH/FSH ratio in both infertile women (r= 0.005, p= 0.957) and the controls (r=0.175, p=181).

4.8 Relationship between Age and BMI

Correlation between Age and BMI showed no significant correlation in infertile women (r=0.161, p=0.079) while there was a positive correlation between age and BMI in controls (r=0.312, p=015) fig.4.7

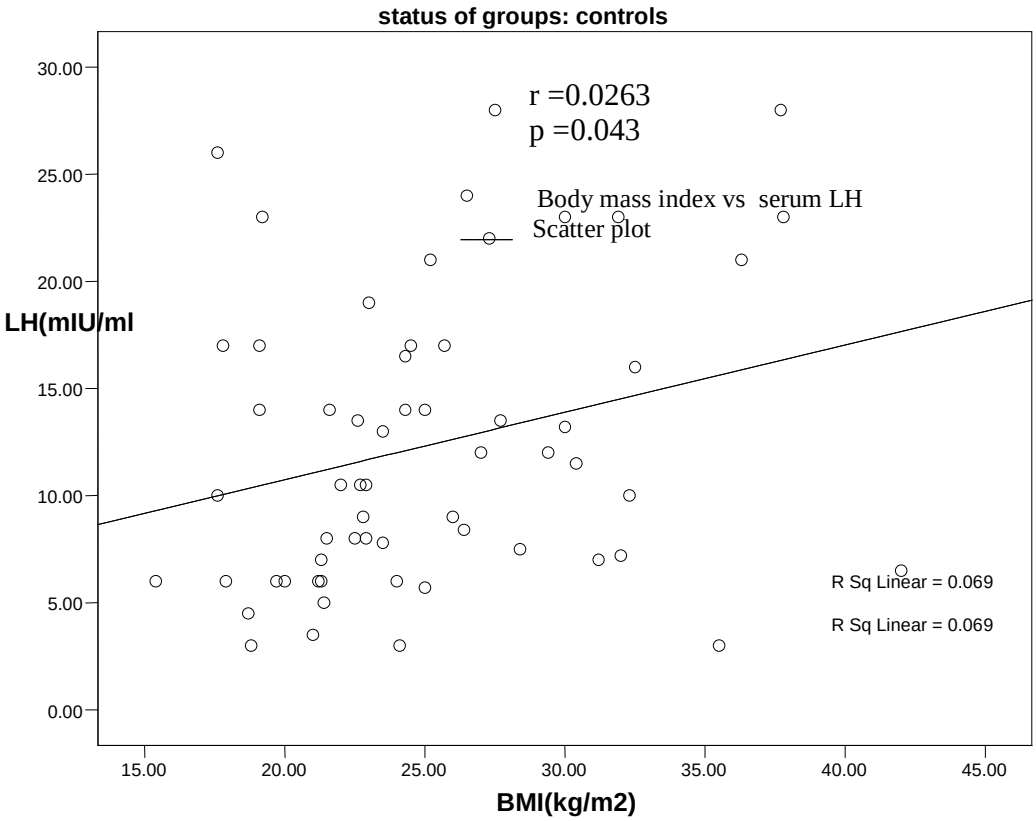


Fig.4.6: Scatter Plot- Showing Relationship between Body mass index and serum LH in controls.

The Line shows positive correlation between Body mass Index and serum LH in controls.

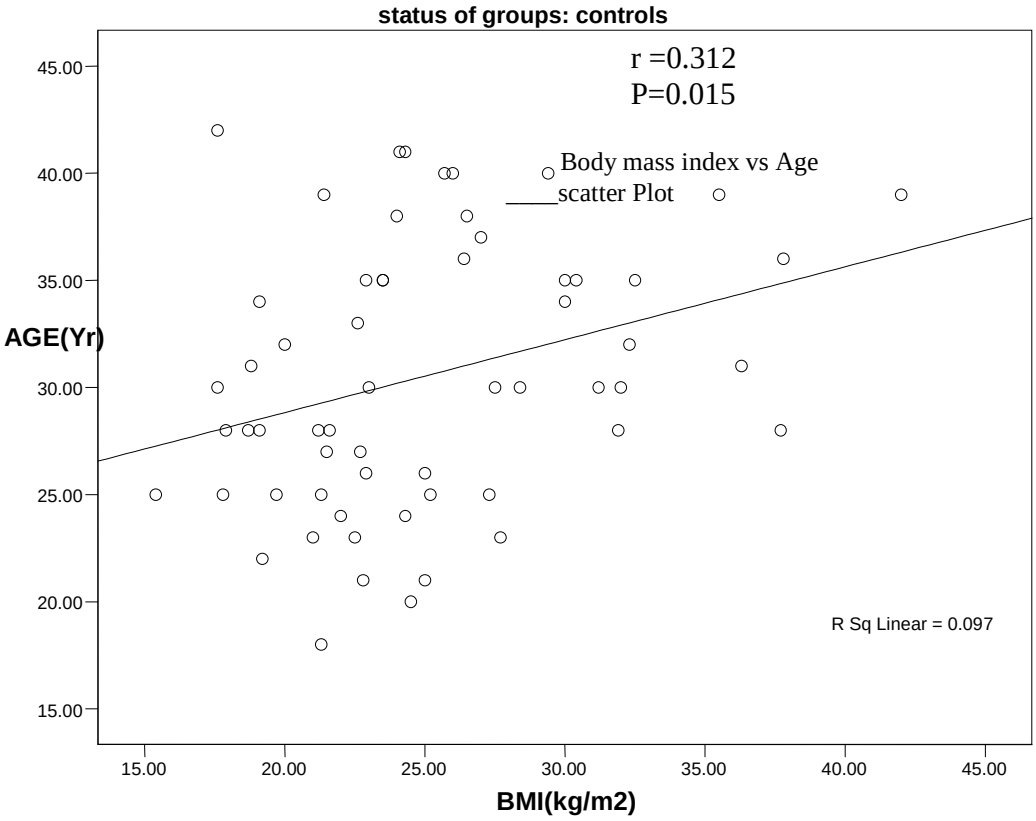


Fig.4.7: Scatter Plot Showing Relationship between body mass index and age in Controls.

The Line shows positive correlation between Body mass Index and Age in Controls.

CHAPTER FIVE

5.1 DISCUSSION

Reliabilty performance characteristics such as accuracy, precision and sensitivity of the serum reproductive hormones assay techinique have been assessed using diagnostic reagents kits supplied by Syntron Bioresearch, Inc. 2774 Loker Avenue West. Carlsbad, California. USA

The evaluation of the methods used in this study for the measurement of the serum reproductive hormones(LH, FSH, prolactin, progesterone and oestradiol) shows acceptable levels of precision and good linearity with a significant correlation of r = 0.970, r = 0.999, r = 0.973, r = -0.826, r = -0.881 respectively and an acceptable coefficient of variation (CV) limits that ranges between 5.9% – 8.0% (within-runs) and 6.2% – 9.8% (between-runs) for LH, 7.2% - 8.4% (within-runs) and 5.5 %– 9.0% (between-runs) for FSH, 3.1% –7.5% (within-runs) and 6.9% – 9.3% (between-runs) for prolactin, 3.1% – 7.5% (within-runs) and 6.9% – 9.5% (between-runs) for progesterone, 2.1% – 5.1% (within-runs) and 2.7% – 10.1% (between-runs) for Oestradiol. Thus the method has an acceptable level of precision . The results also demonstrate that the assays were more

precise at high reproductive hormone concentration because of the lower CV for both with-runs and between-runs precision profiles.

The clinical characteristics of the controls and infertile women recorded in this present study showed a mean age of 30.5 (18-42) years and a mean age 31 (19-45) years respectively, which are within the normal reproductive age of women in this environment.

This was similar to the findings of chigumadzi *et al*, (1998) who in their study population at King Edward VIII Hospital, Durban, found that the mean age of black, infertile women was 31 years, while in a study by Wittemer *et al.*(2000), on women referred for IVF in the USA, the women's mean age was 33.07 years.

It has been found that more of the infertile women (62.5%) and (50%) of the controls in this present study fell in the age group 25-34 years. This is in keeping with the study by Cates *et al*,(1985) in which they found the majority of infertile women in an African setting to fall in the same age group.

The mean duration of infertility of the infertile women in this present study was 5year. This differs from the findings of Cates et al (1985) in which over 2/3 of infertile couples in developing countries only seek medical attention after trying to conceive for more than 2.5year. On the contrary,

about half of couples in developed countries seek evaluations after trying for less than 2years. The possible explanations in Zaria is that most of the couples only present after several years of seeking the assistance of traditional/spiritual healers and general practitioners and therefore present at a later age.

It has been found in this present study that among the infertile women (31.7%) had primary infertility and (68.3%) had secondary infertility. This is attributed to the fact that the major cause of infertility in African women is due to damage to fallopian tube function which usually occurs following pelvic inflammatory disease, post abortal sepsis and peuperal complications.

The results of anthropometric measurements obtained in the present study show that weight and body mass index(BMI) were higher in infertile women than in controls and this differences were statistically significant . These findings were similar to other published reports (Rogers and mitchel 1989, Hantz, *et al* 1995, Bates, G.W, 1999) where the increase in weight and BMI indicated either overweight or obesity. This may probably result from a chronic imbalance between energy intake and energy expenditure. Cultural factors, such as the increased availability of high caloric/energy dense food, or the decreased need for physical activity,

contribute to its development, and their influence is amplified by genetic predisposition. Obesity causes abnormalities of sex hormones in women of reproductive age leading to oligo-ovulation, anovulation , subfertility and increased risk for miscarriage, both spontaneously and after infertility treatment.

There are also an increase risk for amenorrhea, oligomenorrhea, and menorrhagia due to ovulatory dysfunction.

It has been observed in the present study that in the 120 infertile women studied, the mean serum prolactin, LH, and LH/FSH ratio were significantly higher in infertile women than in controls. Mean serum progesterone and oestradiol were found to be lower in infertile women than in controls and FSH was not significantly different in both the infertile women and controls. According to Guzick,(2004) , a combined elevation of LH and LH/FSH ratio with low or normal FSH and low oestradiol are seen in association with polycstic ovarian syndrome (PCOS). It was also observed from this study that 46.6% of the infertile women had elevated LH, LH/FSH ratio, low progesterone and oestradiol which are biochemical evidence of androgen excess an indication that infertility in these women may be due to PCOS. But suprisingly no records of PCOS cases were recorded among the 550 infertile women studied by Emokpae, *et al* (2005) even though it was well

documented that PCOS is one of the commonest endocrine disorders among African women with infertility. And again there were no records of anthropometric data from their study to know the incidence of obesity among these women since obesity is one of the major predisposing factors in PCOS .

However the finding in this study was similar to the one reported by Steven, M. (2003) in South Africa who reported 45.8% of the 150 infertile women to have PCOS.The exact cause of PCOS is unclear but it is thought to be caused by hypothalamic disorder. Polycystic ovary syndrome is a heterogeneous clinical entity characterised by signs and symptoms of hyperandrogenism and anovulatory disorders often associated with infertility and obesity. Relatively low FSH concentration and disproportionately high LH with a ratio of LH to FSH of greater than 3 are common in PCOS. This reflects an increase in androgen production by theca cells which is LH dependent and is associated with anovulation. It is also argued that the probable cause of the anovulation may be insufficient FSH. Follicles in the ovaries of women with PCOS do not mature fully and the granulosa cells in these arrested follicles are low in number and aromataze activity. Therefore oestradiol production by this follicles is limited and hence the low oestradiol levels observed in this study.

The high serum prolactin observed in this present study signifies hyperprolactinaemia. It has been observed in the present study that in the 120 infertile women studied, hyperprolactinaemia was found in 50% of cases.This differs from the findings of Emoakpe *et al* (2005) who recorded 8.5% with 550 infertile women in Kano. However, the finding in this study is similar to the findings reported by Kuku *et al* (1992) in Lagos in which 48.9% of the infertile women had hyperprolactinaemia. Prolactin is involved in the regulation of gonadal function. It stimulates the generation of LH receptors in the gonads. In the female this effect occurs in the ovarian corpus luteum. Hyperprolactinaemia suppresses gonadal function by short-loop negative feed-back inhibition of LH release. It also reduces GnRH secretion and interferes with ovulation.Therefore, in addition to the galactorrhoea, infertility and amenorrhoea often occur (Laycock & Wise, 1996, Cramer *et al.*,2003).

The commonest causes of hyperprolactinaemia includes stress, nonfasting sample, excessive exercise, trauma, renal failure, and cirrhosis. Postictal patients also develop hyperprolactinaemia within 1-2 hours after a seizure. Many common medications cause hyperprolactinaemia e.g Dopamine-receptor antagonists (phenothiazines, butyrophenones, thioxanthenes, risperidone, metoclopramide, sulpiride, pimozide), Dopamine-depleting

agents (methyldopa, reserpine). Others includes (isoniazid, danazol, tricyclic antidepressants, monoamine antihypertensives, verapamil, oestrogens, antiandrogens, cyproheptadine, opiates, H_2-blockers [cimetidine], cocaine). Hyperprolactinaemia may also occur as a consquence of polycystic ovarian syndrome and prolactin secretory tumour(Turex, 2003)

Another potential cause of hyperprolactinaemia is macroprolactinaemia. Macroprolactinaemia is the apparent increase in serum prolactin without symptoms. In this condition, serum prolactin molecules can polymerise and subsequently bind to immunoglobulin G (IgG).

This form of prolactin is unable to bind to prolactin receptors and exhibits no systemic response. In the asymptomatic patient with hyperprolactinaemia, this condition should be considered.

It was observed from the present study that hyperprolactinaemia has the highest incidence of all the hormonal abnormalities and it also coexists with some other hormonal abnormalities such as polycystic ovarian syndrome, hypergonadotropic hypergonadism and hypergonadotropic hypogonadism as observed in this study. This may suggest the presence of multiple endocrine disorders in some of the infertile women .

Hyperprolactinaemia may occur on it’s own as a cause of hypogonadotropic hypogonadism in the infertile women or it may occur as consquence of

polycystic ovarian syndrome.The hyperplactinaemia observed in this study may be as a result of polycystic ovarian syndrome and probably as a result of stress associated with infertility in this group of patients.

It has been observed in this present study, that 4.2% of the infertile women had elevated LH, FSH and low progesterone and oestradiol which characterised hypergonadotropic hypogonadism or primary ovarian failure (POF) . This was similar to the findings of Emoakpe *et al* (2005) who found 4.7% of the infertile women to have hypergonadotropic hypogonadism. Female hypergonadotropic hypogonadism, occurs as a result of primary ovarian hypofunction an indication that failure to conceive may lie in the ovary.

Chemotherapy, radiation therapy or both are frequently acquired causes of ovarian failure. Autoimmunity may also play a role in some cases of POF without the presence of specific autoimmune diseases. In such cases, antibodies specifically attack the cells that secrete reproductive hormones thus causing ovarian failure.

Other causes of primary female hypergonadotropic hypogonadism include Turner's syndrome (45 XO) – phenotypically female with infantile sexual development and ovaries containing no follicles , menopause, testicular feminisation (androgen insensitivity - phenotypically female,

genetypically male) , LH or FSH receptor mutation, XX or XY gonadal dysgenesis and polycystic ovary disease.

The finding of the present study also shows that 1.7% of the infertile women has elevated LH, FSH and progesterone which signifies hypergonadotropic hypergonadism . This finding was similar to the finding of Emokpae. M.A *et al* (2005) who found 1.1% of the infertile women to have hypergonadotropic hypergonadism.

It was also observed in this present study that of 120 infertile women studied 1.7 % has low LH, FSH, and progesterone this signifies hypogonadotropic hypogonadism . This differs from the findings of Emoakpe *et al,* (2005) who found 3.5% from 550 infertile women studied to have hypogonadotropic hypogonadism.

The genetic causes of hypogonadotropic hypogonadism includes Kallman's syndrome (Anosmia and Gonadotrphin releasing hormone deficiency), isolated LH or FSH deficiency, PROP1 gene mutations that lead to absence of some pituitary hormones. Secondary causes of hypogonadotropic hypogonadism includes celebral tumours pituitary tumours, astrocytoma, head trauma , cerebral infections celebral radiation, chronic systemic disease and malnutritions . Other causes includes hyperprolactinaemia diabetes mellitus and marijuana use.

It has been observed in this present study that out of 29 infertile women who were in their luteal phase, luteal phase deficiency was found in 65.5% of the study group and 59.3% of this group were found to coexist with hyper prolactinaemia. This differs from the findings of Emokpae, M.A *et al* who found 12.8% of the infertile women who had hyperprolactinaemia but normal LH, FSH and low progesterone. Luteal phase deficiency occurs when there is deficient progesterone secretion by the corpus luteum. The relationship between hyperprolactinaemia and luteal phase deficiency was first documented by mcNatty and colleagues in 1974 and they found that neutralisation of prolactin in culture medium with specific antibody or addition of prolactin over threshold leads to diminished daily progesterone production *in vitro*. In another study induced mild hyperprolactinaemia shows reduced Luteal phase progesterone. These findings corroborate the theory proposed by Bahamondes, *et al* that luteal phase defficiency is a part of natural sequence of events between normal ovulatory and amenorrhoea that occurs in women with progressive hyperprolactinaemia. It therefore means that luteal phase defficiency is a common disorder in infertile women with hyperprolactinaemia and this may explain the luteal phase deficiency observed in this study.

The findings in the present study demonstrate that there were no

correlations between BMI and all the reproductive hormones in infertile women, while there were positive and significant correlations between BMI and serum LH in controls. However the serum level of LH increases as the BMI increases, and that this type of association does not exist among the infertile women.This differs from the findings of De pergola, *et al* 2006, where overweight and obese fertile women have lower LH levels in the early follicular phase. This may probably be due to a possible direct inhibitory effect of body mass on gonadotropin production. In this study the majority of the fertile women(56.6%) were within nomal weight and probably the direct inhibitory effect of the body mass on gonadotropic production may have no effect or it may rather have stimulating effect on the gonatropin production; this may be the reason for the direct correlation observed between BMI and LH.

There were no significant correlations between age and BMI in infertile women , while there were positive and significant correlations between age and BMI in controls. This suggests that BMI increased with advancing age among the controls and this relationship was absent among the infertile women.

5.2 CONCLUSIONS.

1.The mean levels of serum LH, Prolactin and LH/FSH ratio were found to be significantly higher while progesterone and oestradiol levels were significantly lower in the infertile women than in the controls while 28.3% of them were found to have a normal hormonal profiles.

2.Hormonal abnormalities were seen in 71.7% of infertile women comprising 33.3% polycystic ovarian syndrome with hyperprolactinaemia, 13.3% luteal phase deficiency with hyperprolactinaemia , 13.3% polycystic ovarian syndrome, 4.2% Hypergonadotropic hypogonadism, 2.5% luteal phase defficiency, 1.7% Hypergonadotropic hypogonadism with hyperprolactinaemia, 1.7% Hypogonadotropic hypogonadism and 1.7% Hypergonadotropic hypergonadism.

3.Hyperprolactinaemia was found in 50% of infertile women studied and coexisted with some other hormonal abnormalities .

4.Polycystic ovarian syndrome was found in 46.6% of infertile women studied.

5.There was positive and significant correlation between BMI and serum LH in controls.

6.Even though some information exist on hormonal levels in Nigerian infertile women this is scanty in Zaria, Northern Nigeria.

7.The results of this study indicate that hormonal disorders may be responsible for some cases of women with infertility. These results also suggest that hormonal disorders that occurs in infertile women in Zaria, Nothern Nigeria may be treatable.

5.3 RECOMENDATIONS

Estimation of serum levels of LH, FSH, progesterone, Prolactin and oestradiol should be part of routine investigation of infertile women in Nigeria in order to assess the aetiology of hormonal abnormalities and determine the treatable groups; these results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

REFERENCES

1.ACOG Technical Bulletin-Managing the anovulation state:medical induction or ovulation.No.197 *Int. J. Gynaecol Obstet1994; 47: 306-312.*

2.American fertility foundation. What you should know about infertility.*Contemporary ob/gyn 1980; 14:101*

3.Bauman, JE, Basal body temperature: unreliable method of Ovulation detection. *Fertile steril 1981; 36: 729-756*

4.Dyer S.J. Abrahams N. Hoffmann, M and Vander Spuy, Z.M. Men leave me as I cannot have children. Women experiences with involuntary childlessness. *Human reproduction, 2002a;17:.1663-1668*

5.Dyer S.J. N. Hoffmann, M and Vander Spuy. 2002b, Infertility in South Africa women reproductive health knowledge and treatment–seeking behavior for involuntary childlessness. *Human reproduction,2002b;17:1657-1662.*

6.Ferrin, M. Jewele wicz, R. and Wamen M. The menstrual cycle. Physiology reproductive disorders and infertility. New York: Oxford University press,1993.

7.Fox H. Buckley CH. Pathology of female infertility. In; Anthony pp, 'Macsworu RNM (eds). Recent advances in Histopathology (No.11) Churchhill Livingstone, Edinburgh 1981; 119-134.

8.Heymfield, S.B. Baumgartner, R.M. and Pan S. 1999 Nutritional assessment malnutrition in health and disease Ed. By M.E Shils, J.A Olson, M. Shike and A.C. Ross. 9th ed. Philadelphia: Linppincott. Williams and Wilkins.

9.Hillis SD, Joesoef R Machbanks PA, Wasseabeit JN, Westron L: Delay care of pelvic inflammatory disease as a risk for impaired fertility ,*Am J Obstet Gynecol 1993: 168: 1503-1505*

10.Hull MGR, Glazemer CMA, Keller NJ, Conway DI, Foster et al: population study of causes. Treatment and outcome of pregnancy: *Brt Med J 1984: 291: 190-299*

11.Iris. A, Kawuwa MB. Habu SA, Adebayo A. Prolactin levels among infertile women in Maiduguri Nigeria. *Tropical journal of Obstetric and Gynaecology 2003; 20: 97-100.*

12.Katro, B.N.: impaired fertility caused by endocrine dysfunction in women. *Endocrinology clinics of North American, 2003; 32: 573-592.*

13.Ladipo OA: The epidemiology of infertility. DOKITA 1987;16(1):13-19Lee R.D Nieman, D.C 2003. Nutritional assessment 3rd ed. Boston. Mc Graw Hill.

14.Matijkg, Ladipo,The Johns Hopkins programme for international education in Gynaecology and Obstetrics 1984: 193.

15.Nandi E, Ory SJ, Herrmann R, O Brien PS, Melton III LJ: fertility after ectopic pregnancy. *Brt Med J 1984: 291 :190-299*

16.Okonofua R. infertility and women reproductive health. In Africa (editorial) *Afr.J. Reprod. Health 1999; 3: 1-3*

17.OnG Sc, Rahman SS. Induction of ovulation. A review. Singapore *J. obstet. Gynaecol 1985; 16: 131-147*

18.Ory, S.J, The differential diagnosis of female infertility. In principles and practices of endocrinology and metabolism. Lipincott, Philadelphia 1990:789-795.

19.Pilgrim, T.C. 2001. The hidden of infertility in African American community. Available from http://www.ferre.org/folit ext/hiddent.ltml.

20.Rebar R.W. Kenigsberg D; Hoogen GD, The normal menstrual cycle and the control of ovulation. In: Becker KL (Ed). Principles and practice of endocrinology and metabolism. Lippincott endocrinology, Philadelphia, 1990; 778-787.

21.Rowel PJ: Epidemiology of infertility. In: Advances in Gynaecologic Endocrinology : proceedings of 1st congress of international Society of Gynaecological Endocrinology(cds AR Genazzani. F petraglia. A Volep. F pacchinetti I Parthenon. Carnforth. Lancashire. UK 1988

22.Sciarra JJ: Infertility: An International Health Problem. *Journal Gynaecol Obstet (special issue) 1994: 46(2): 155-163*

23.Sciarra JJ: Selected topics of international interest.1988. In: Advances in Gynaecology and Obstetrics(eds P Belfort, JA Pinotti, TKAB Esks) P. 1-15. the proceedings of the XI World Congressof Gynaecology Obstetrics,Rio de Janeiro,October,1988.

24.Sharara, Fil McClamrock, H.B . Difference in In vitro fertilization (IVF) out come between white and black women in an inner city university based IVF program. *Fertility and sterility;2000;73(6):1170-1173*

25.Ujah I.A Lynch. CB, McCune G. Ovulation induction in Clomiphen resistance polycystic ovarian syndrome. By pure FSH. (Uro fillitrophin): a comparism of two regimens. *Tropical journal of Obstetric and Gynaecology. 1991; 9: 35-37*

26.Willocks. J. and Neilson J.P 1991 Obstetric and Gynaecology 4th ed. Edinburgh: Churchill Livingstone.

27.World Health Organisation (WHO): Infection, Pregnancies and Infertility: Perspective on Prevention. *Fertile Steri;l 1987:14: 964-966.*

APPENDIX I

CONSENT FORM

Serial No……..

Hospital No……..

Age……..

Consent Form to participate in a study

The research is designed to assess the pattern of reproductive hormones in women with infertility in Zaria. Blood will be required for the study.

The results obtained will be treated with confidentiality.

You are free to participate or decline in this study without any consequence.

This study will hopefully improve your present health status and that of others in the future.

If you desire to participate in the study please kindly sign the attached form below.

Thank You.

APPENDIX II

INFORMED WRTTEN CONSENT

I…….....of……

……...agreed to participate in this study of assessment of the pattern of reproductive hormones in women with infertility in Zaria. The full procedure and the probable benefits of the test were fully explained to me.

I was informed that a sample of my blood will be collected for the test. If I so wish, the test results will be communicated to me in confidence.

I made this consent willingly without being subjected to any pressure.

Participant Name…….....

Signature……

Witness Name……

Signature……...

Researchers Name…….

Signature……

APPENDIX III

QUESIONAIRE ON PATTERN OF REPRODUCTIVE HORMONES IN WOMEN WITH INFERTILITY IN ZARIA NORTHERN NIGERIA

Please read the following carefully.
Before any detailed study of your problems can be undertaken,it is important that you are familiar with all the pertinent facts. Therefore it is to your benefit that you answer the following question honestly.
You are assured that the this confidential questionnaire as part of your case history wil be held in the strictest confidence, according to the ethics of the medical profession.

SECTION A MEDICAL HISTORY

Serial no: .....

Hospital no: .....

Age in years.....

Ethnic Group.....

Educational Status:.....(a)primary (b)secondary (c)tertiary

Occupation: .....

Duration of infertility in years.....

Age at menarche.....

Date of last child birth if any……..

menstrual History (a) Regular (b)irregular

Menstrual phase (a) follicular (b) ovulatory (c) Luteal (d)Amenoric

Types of infertility (a) Primary (b) Secondary

History of Galactorrhoea: (a)Absent (b) Present

History of** Hirsutism* ***(a) Absent (b) Present

His tory of use of contraception....(a)No (b)Yes

History of past drug treatment: (a) No (b) Yes

If yes what drug treatment ……..

Any family history of infertility?. (a)No (b)Yes

SECTION B. PHYSICAL EXAMINATION

Weight..... (Kg)Height..... (m)

Body mass index (BMI).....(kg/m^2)

Blood Pressure.....

Development of Secondary sexual characteristics:

(a)well developed (b)poorly developed

Galactorrhoea: (a)Demonstrable (b)Not demonstrable

Hirsutism.: (a)Demonstrable (b)Not demonstrable

Virilism: (a) Demonstrable (b)Not demonstrable

SECTION C LABORATORY RESULTS

Progesterone…….(ng/ml)

Oestradiol……(pg/ml)

Prolactin……(ng/ml)

Luteinizing hormone (LH)……..……(mIU/ml)

Follicle Stimulating Hormone(FSH)……...……..(mIU/ml)